TWO RARE INSTANCES OF CARDIOVALVULAR DISEASE PRESUMPTIVELY SYPHILITIC IN ORIGIN

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In a report of two cases of cardiac gummas,[1] acquired tertiary syphilis of the heart [2] is considered synonymous with gummatous myocarditis, localized or diffuse. This is in conformance with current concepts. The characteristics of the lesions are definite, and the pathologic diagnosis is generally a simple one.

Two additional cases were recently encountered which lack the accepted diagnostic criteria of gumma, although the collected evidence points to a presumptive diagnosis of tertiary syphilitic heart disease.

This raises a question as to whether the gummatous lesion represents the sine qua non of cardiac syphilis. Enlightenment on this issue may be obtained by analogy with aortic syphilis. Conner,[3] in a masterful survey of the development of knowledge of cardiovascular syphilis, described the bitter and finally successful "struggle for the recognition of the specific nature of syphilitic aortitis." Yet, even today the diagnosis of syphilitic aortitis is at best presumptive and not absolute, since the lesion is usually not gummatous and the specific organism has only rarely been recognized in it.

The two cases now reported present unusual lesions of the mitral valve. As far as can be determined, lesions of the same type have not been described hitherto. Their nature together with certain points of similarity in the findings in proved cases of syphilitic heart disease reported in the literature suggests a clue as to their etiology.

REPORT OF CASE 1

A 51 year old Austrian was admitted to the medical service of Dr. George Baehr on May 1, 1931, complaining that he had had a nocturnal cough productive of greenish sputum for four weeks. Five days previous to admission he was seized with an agonizing and suffocating sense of constriction in the chest. From

1. Sohval, A. R.: Arch. Path., to be published.

2. Exclusive of aortitis with commissural involvement and omitting Virchow's type of diffuse myofibrosis (nondiagnostic).

3. Conner, L. A.: J. A. M. A. **102**:575, 1934.

then on there were moderate difficulty in breathing, "tightness" in the chest and occasional vomiting.

He had pneumonia in childhood, and at the age of 20 years he suffered from gonorrhea and arthritis.

The patient was acutely ill. The temperature was 101 F.; the pulse and respiratory rates were, respectively, 44 and 28 per minute. The left pupil was larger than the right; both were round; both reacted sluggishly to light and were fairly active in accommodation. Crackling râles were auscultated throughout the lungs and were most numerous at the bases posteriorly. By percussion, the heart was found considerably enlarged to the left, the apex being in the sixth left intercostal space in the anterior axillary line. A diastolic murmur was present along the left border of the sternum in the third and fourth intercostal spaces. The rhythm was regular except for many interpolated beats consisting of only one sound. Pulsations corresponding to these could be seen over the region of the internal jugular veins but could not be palpated over the radial artery at the wrist. The pulsations in the latter were of Corrigan's type and equal. By percussion the liver was found to extend 1 fingerbreadth below the free border of the ribs. An old penile scar was present. On the left calf was a superficial flat scar about 3 cm. in diameter with a pigmented border. Neurologic examination disclosed nothing except a positive Hoffmann sign on the left, absence of the plantar and cremasteric responses and pupillary changes as noted.

The Wassermann and Kahn reactions of the blood were negative. The spinal fluid was normal cytologically and serologically (the Wassermann reaction and colloidal gold test were negative). The systolic blood pressure varied between 156 and 190 mm. of mercury, and the diastolic, between 68 and 84. Hemoglobin was 76 per cent. There were 10,000 leukocytes per cubic millimeter of blood, of which 90 per cent were polymorphonuclear neutrophils. The blood contained 27 mg. of urea nitrogen per hundred cubic centimeters. The urine contained a moderate amount of albumin and occasional leukocytes.

An electrocardiogram showed complete auriculoventricular dissociation. The auricular rate was about 110 per minute and the ventricular rate about 58. There was deep inversion of the T waves in all leads. Myocardial damage was suggested by the tracings.

On the third day after admission the patient suddenly lapsed into stupor and died on the following day.

The final diagnosis was syphilitic aortitis, aortic insufficiency and complete heart block.

Autopsy.—The cardiac findings are given in detail. The heart (fig. 1 *A*) was considerably enlarged and weighed 500 Gm. There was no evidence of pericarditis. The right auricle and ventricle were slightly dilated. The tricuspid and pulmonary cusps were normal. The root of the pulmonary artery was normal.

The left auricle was enlarged and somewhat hypertrophied. Its endocardium was thickened and whitened. The posterior mitral leaflet was normal. The anterior mitral leaflet was whitened, and its upper two thirds definitely thickened. This portion felt cartilaginous. When this leaflet was split open it was found to measure 4 mm. at the auricular attachment. The cartilaginous structure was seen to continue upward behind the auricular wedge to become continuous with the root of the aorta, on the one hand, and with the septum fibrosum, on the other. The left ventricle was somewhat hypertrophied and considerably dilated. The posterior papillary muscle appeared to be atrophic and showed hemorrhages at its apex. The anterior papillary muscle was of normal size but also contained hemorrhages at its apex. The outflow tract was decidedly elongated.
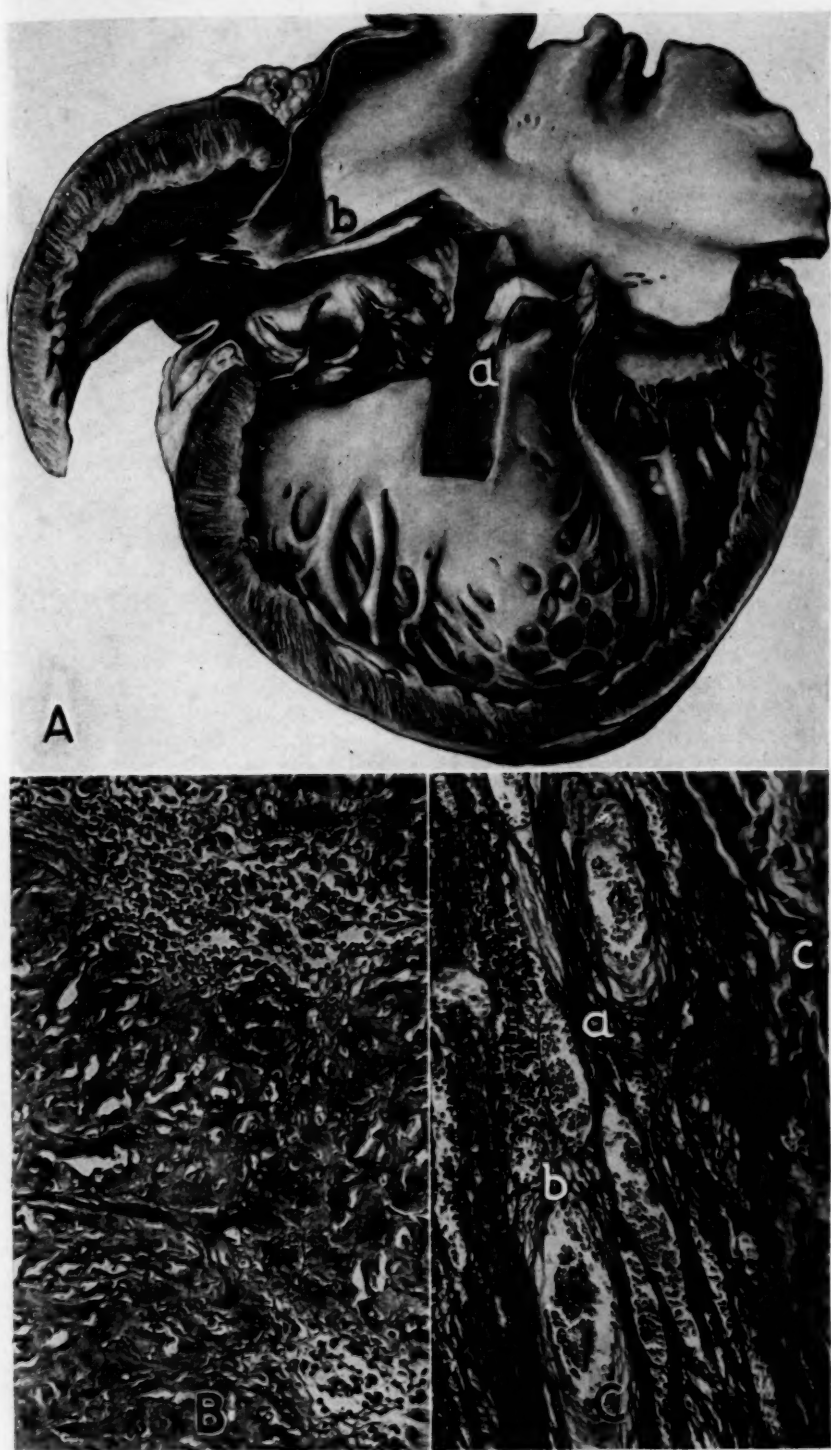
Fig. 1 (case 1).—*A*, view of the left side of the heart showing extension of the dense cartilaginous-appearing lesion into the contiguous membranous septum (*a*) and anterior mitral leaflet (*b*). *B*, fibrosa of the anterior mitral flap under high power magnification, showing perivascular foci of lymphocytes, plasma cells and large mononuclear cells in a dense hyaline stroma. *C*, the same structure on the auricular aspect under high power magnification, showing hyperplasia of the elastica (*a*), numerous vessels (*b*) and diffuse infiltrations with round cells. The fibrosa layer is at *c*. Both *A* and *B* show MacCallum's stain for elastica and Van Gieson's stain. Magnification, 16 mm.; × 200.

The aortic cusps were of usual size. The right cusp showed a rounded thickening occupying approximately 1 cm. of the middle portion of its free edge. The right posterior commissure was somewhat fused. The posterior leaflet showed a rounding and shortening. The posterior extremity of the left leaflet showed a similar lesion, and the left posterior commissure was the site of a moderate separation (2 mm.). The endocardium over the undefended space was thrown up into rugosities. These prominences felt somewhat rubbery, and the undefended space was opaque to light. The rugosities over the endocardium of this space were continuous with a similar lesion on the ventricular surface of the anterior mitral leaflet and in the region of the left posterior commissure. The lesion continued through the separation of this commissure and could be seen very markedly within the pockets of the component cusps. The sinus pocket of the right cusp also showed striations and rugosities which, however, were less marked. The right-left commissure showed a separation approximately 1 mm. in width.

The aorta measured 8 cm. at its root and showed mild sclerotic plaques. Coronary arteries and their branches showed discrete mild atheromatous changes without narrowing.

Microscopic Examination.[4]—Aorta: The ascending portion above the root presented only mild alterations. A slight fibro-elastic proliferation was present in the intima. There were many capillaries surrounded by small areas of fibrous tissue in the outer half of the media. The vasa vasorum of the adventitia were thickened by a hyperplastic intimal process with some narrowing of the lumens and perivascular accumulations of plasma cells and lymphocytes.

Mitral and Aortic Valve: The sinus wall of the posterior aortic cusp, containing the root of the aorta and the annulus fibrosus of the valve, was liberally involved by foci of lymphocytes, fibroblasts, large mononuclear and plasma cells and capillaries, which continued down to implicate the ring and base of the valve cusp and the dense fibrous tissue behind the annulus. From here this inflammatory process, set in whorls of connective tissue, extended through the intervalvular fibrous layer into the fibrous layer of the anterior leaflet of the mitral valve. The latter structure was therefore markedly broadened. The aortic cusp was normal (except for the inflammatory lesion at the base). Its tip was expanded into a noninflammatory, avascular, fibro-elastic, bulbous formation. Superimposed on the sinus wall was a well developed fibrous proliferation continuous with that of the intima of the aorta just described.

Anterior Cusp of Mitral Valve: The leaflet was markedly and diffusely widened for the proximal two thirds of its length by a dense hyaline connective tissue expansion of the fibrous layer continuous with that described in the intervalvular fibrosa. It was arranged in whorls and was extensively involved by numerous small capillaries (some with well developed elastic coats) and perivascular aggregations of plasma cells, lymphocytes and larger mononuclear cells (fig. 1 B). At each border of the fibrosa there was a condensation of the inflammatory process which formed the base of a band of fibrous tissue proliferation, fairly well supplied with elastica, vascularized, infiltrated, and more prominent on the auricular side, where the vessels were larger and had thicker walls (fig. 1 C). The original auricular and ventricular elastic lamellae tended to be frayed and reduplicated. This chronic inflammatory process likewise infiltrated the tip of the left auricular myocardial wedge. The Levaditi stain was negative.

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4. Blocks of cardiovalvular tissue in this and the following case were cut according to the standardized procedure of Gross, Antopol and Sacks (Arch. Path. **10**:840, 130). Sections were stained with hematoxylin and eosin and in duplicate with Weigert's elastic and Van Gieson's connective tissue stain.

Posterior Cusp of Mitral Valve: The ring and valve were normal.

Tricuspid Valve and Interventricular Septum: The ring and proximal portion of the leaflet contained a generous number of capillaries and round cells. The fibrous septum was markedly thickened by a sclerocellulovascular process identical with that described in the anterior mitral flap. The region of the bundle of His was the site of a more focal and extensive, though similar, involvement with elastic scar tissue and obliterative arteriolar lesions. There were large numbers of fibroblasts. In its midst were seen remnants of degenerated muscle fibers.

Pulmonary Valve: The ring, valve and artery were normal.

Posterior Papillary Muscles: There were extensive areas of deeply staining myocardial fibers of hyaline appearance with loss of nuclei and striations, indicative of massive necrosis. In general, their form was preserved, although in occasional foci they were completely disintegrated. Between the muscle bundles were extravasted erythrocytes and marked infiltrations of leukocytes, almost entirely polymorphonuclear, and arranged in more or less linear distribution. There was a moderate amount of perivascular fibrosis.

Left Auricle: The auricle was normal except for some sclerotic thickening of the endocardium.

Other significant findings at autopsy included congestion and edema of the lungs and congestion of the liver and spleen. Evidence of extracardiac syphilis was not discovered.[5]

COMMENT ON CASE 1

Because of the history of gonorrhea, the cardiac findings, Corrigan pulse, penile scar, pupillary signs and reflex changes, this patient was regarded clinically as presenting syphilitic aortitis with aortic insufficiency. Necropsy not only confirmed this impression but disclosed a lesion of cartilaginous hardness in the mitral valve and septum fibrosum with destruction of the bundle of His. While a lesion in the latter situation might have been surmised from the clinical and electrocardiographic evidence of heart block, the alteration in the mitral valve was a completely unexpected finding since it gave rise to no clinical signs.

Until recently, the existence of proof of syphilis of the mitral valve was denied.[6] To date there are but two authentic cases on record; Friedman[7] and Staemmler[8] each reported one case of mitral syphilis in combination with syphilitic aortitis and aortic insufficiency. The Wassermann reaction of the blood was positive in both. In each case the lesion consisted of a firm thickening of the anterior leaflet by dense inflammatory connective tissue. There were diffuse infiltrations of round cells. Miliary areas of necrosis and young capillaries were present. Giant cells were found only in Staemmler's case. Schmorl[9]

5. The brain was not examined.

6. Breitmann, M.: Gaz. d. hôp. **76**:213, 1903. Steinberg, M. J.: Am. J. Syph. **12**:316, 1928.

7. Friedman, W.: Proc. New York Path. Soc. **24**:24, 1924.

8. Staemmler: Verhandl. d. deutsch. path. Gesellsch. **25**:262, 1930.

9. Schmorl, in discussion of report by Staemmler.[8]

cited Geipel's case of lesions in the mitral valve identical with those in Staemmler's case. Other cases of so-called syphilitic mitral valve disease are most probably instances of associated endocarditis.

In view of the extreme rarity of syphilitic mitral valvulitis, great caution must be exercised in accepting a new case, especially in the absence of coagulation necrosis. However, certain features point strongly to syphilis as the etiologic factor in the instance under discussion.

The aortitis and aortic insufficiency were typically syphilitic. The negative serologic data do not vitiate the diagnosis since the serologic findings in a fair percentage of cases (about 20 per cent) of proved cardiovascular syphilis are negative.

Blocks of cardiovalvular tissue were cut according to the standardized method of Gross, Antopol and Sacks.[10] These included one section taken through the root of the aorta, the posterior aortic cusp and the anterior flap of the mitral valve. From a study of the normal topography in this region (fig. 2), it is not difficult to see how the medial and adventitial lesions of syphilitic aortitis may descend to involve the annulus fibrosus of the aortic valve, the loose tissue behind it, the intervalvular fibrous layer and the fibrosa of the anterior curtain of the mitral valve.

Microscopic examination of this crucial section enabled one to trace the entire process as it extended from the root of the aorta into and behind the aortic annulus, through and alongside the intervalvular fibrosa and into the fibrosa of the aortic flap of the mitral valve. These observations confirm the mode of extension noted by Staemmler[8] in his case. They likewise represent a much later stage than the early, mild process noted subsequently in case 2 (fig. 4 A and B).

The cause for the complete heart block noted during life was found in the densely fibrous and granulomatous lesion in the membranous portion of the interventricular septum involving and destroying the bundle of His. This infiltration had the same gross and histologic characteristics as those noted in the anterior flap of the mitral valve and root of the aorta with which it was continuous. It likewise most probably originated by extension from the root of the aorta.

To the twelve instances of heart block due to cardiac syphilis collected by Major[11] are added those of Handwerch,[12] Holterdorf,[13] de Marval and Vivoli,[14] Cleland[15] and Kux,[16] bringing the total number

10. Gross, L.; Antopol, W., and Sacks, B.: Arch. Path. 10:840, 1930.

11. Major, R. H.: Arch. Int. Med. 31:857, 1923.

12. Handwerch, C.: München. med. Wchnschr. 56:916, 1909.

13. Holterdorf, A.: München. med. Wchnschr. 63:1651, 1916.

14. de Marval, L., and Vivoli, D.: Rev. Soc. argent. de biol. 2:425, 1926; Rev. Soc. de med. int. y Soc. de tisiol. 2:397, 1926.

15. Cleland, J. B.: M. J. Australia 1:540, 1927.

16. Kux, E.: Ztschr. f. Kreislaufforsch. 24:1, 1932.

of recorded cases to seventeen. While typical isolated cardiac gummas involved the conduction system in the vast majority of these cases, the lesion in the instance reported by Vaquez and Esmein [17] closely resembled that found in this case in the predominance of sclerotic and absence of necrotic processes. They accordingly designated the lesion as a sclerogummatous one, despite the fact that giant cells and coagulation necroses were absent.
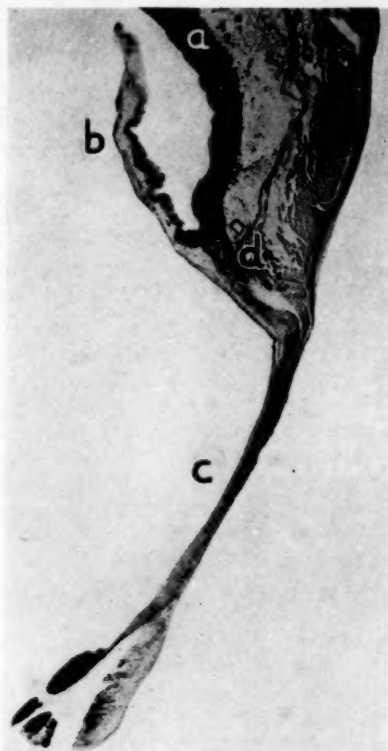

Fig. 2.—Reproduction from Gross and Kugel (Am. J. Path. **7**:445, 1931, fig. 6), showing the topography of the normal aorta (*a*), posterior aortic valve (*b*) and anterior mitral valve (*c*). This indicates the path of extension of syphilis from the root of the aorta through and behind the intervalvular fibrosa (*d*) to the aortic leaflet of the mitral valve. Weigert's stain for elastica and Van Gieson's connective tissue stain were used. Magnification, 75 mm.; × 4.

In the light of the foregoing observations, it is evident that the lesion in the anterior leaflet of the mitral valve in this case originated from a syphilitic process at the root of the aorta and was continuous with an identical lesion in the septum fibrosum. It consisted essentially

17. Vaquez and Esmein: Presse méd. **15**:57, 1907.

of a well scarred granuloma, presumably syphilitic. The absence of positive Levaditi stains does not render this less likely, since such is the rule even in authentic cases. The close resemblance of the valvular lesion to that noted by Friedman [7] and Staemmler [8] (differing essentially in the absence of coagulation necrosis and the predominance of fibrosis) is very suggestive. The striking similarity of the lesion in the conduction system to that observed by Vaquez and Esmein [17] further strengthens the probability that the condition was syphilitic.

The advanced sclerotic condition of the lesion lacking coagulation necrosis and giant cells may indeed signify that a previously gummatous infiltration is now undergoing healing and scarring. On the other hand, this type of lesion may simply represent syphilitic invasion of the fibrous structures of the heart (valve and fibrous septum) from a contiguous process at the root of the aorta.

<div align="center">REPORT OF CASE 2</div>

A 20 year old white woman, unmarried, was admitted to the hospital under the care of Drs. H. Abramson and B. S. Oppenheimer on March 29, 1930, complaining that she had had "bronchitis," dyspnea, hemoptysis and pretibial edema for three weeks. Two years prior to admission she was found to have a "leaking" heart. Serologic examination of the blood and spinal fluid had been performed and the results reported as negative. She had a paratyphoid infection at the age of 4 and influenza during the pandemic.

She was admitted to the hospital in an almost comatose condition. The temperature was 98.6 F.; the pulse rate was 128 and the respiratory rate 18 per minute. She was dyspneic and pale. Marked pulsation of the peripheral arteries of the extremities and neck was apparent. The left internal jugular vein was prominent. There was edema of the legs, thighs, sacrum, back, left hand and left breast. The pupils were dilated and did not respond to light. There were physical signs of an effusion in the left pleural cavity. Râles were heard diffusely throughout both lungs. The heart was enlarged; its action was regular. Systolic and diastolic murmurs were heard over the aortic area. The liver was tender and palpable as far as the umbilicus. She continued to grow worse and died several hours after admission to the hospital.

The systolic blood pressure had been 100 and the diastolic 0. Hemoglobin was 60 per cent, with 2,560,000 erythrocytes and 30,000 leukocytes per cubic millimeter of blood. The differential count was 77 per cent polymorphonuclear cells and 23 per cent lymphocytes.

The final diagnosis was myocardial decompensation, chronic cardiovalvular disease and aortic insufficiency.

Autopsy.—The pericardial cavity contained approximately 100 cc. of clear amber-colored fluid. The heart (fig. 3) weighed 500 Gm. It was much elongated and presented a marked dilatation of both ventricular cavities. The right auricle was moderately dilated. The tricuspid valve, pulmonary cusps and pulmonary artery were normal.

The left auricle was slightly dilated; its endocardium was markedly whitened. The aortic leaflet of the mitral valve was firm and thickened. The chordae tendineae had normal insertions into the free margin of the cusp. The posterior papillary muscle showed marked thickening of its endocardium and had a dense

whitish appearance. The left ventricle was moderately hypertrophied. The apex of the left ventricle, near the septum, contained an adherent firm thrombus. The outflow passage had a dense white endocardium, and its myocardium was scarred.

The ventricular surface of the aortic leaflet of the mitral valve was the seat of a diffuse deposit of firm consistency, pearly color and finely striated surface. This deposit extended up the outflow passage over the membranous septum to the base of the right and posterior aortic cusps, where it extended for a very short distance on these cusps. There was a striking defect of the right cusp in the form of a complete absence of the entire half adjacent to the posterior cusp. Only the line of insertion of this part of the cusp remained. It was situated 1 cm. below the original annulus fibrosus, which was still visible. The posterior cusp, aside from the moderate thickening and a small deposit of material similar to that

Fig. 3 (case 2).—View of the left side of the heart showing syphilitic aortitis (*a*), loss of substance of the posterior portion of the right aortic cusp (*b*) and extension on to the endocardium over the membranous septum (*c*) and the aortic leaflet of the mitral valve (*d*). An adherent mural thrombus is shown at the apex (*e*).

already described, presented nothing remarkable. This was also true of the left cusp. The aortic valve commissures appeared normal except in the region of the defect, where there appeared to be some slight separation.

Rising from the region of the posterior right commissure there was a more or less circular, closely ridged elevation about 2.5 cm. in diameter, which appeared to be continuous through the absent portion of the cusp with the striated deposit on the membranous septum and the anterior mitral leaflet. The remainder of the aorta showed practically no change. The coronary orifices were normal. The left anterior descending and left circumflex coronary arteries were patent throughout, although moderately sclerotic.

Microscopic Examination.—Aorta: There was marked fibro-elastic prolifera-
tion of the intima.   The architecture of the media was tremendously distorted.
There were innumerable irregular scars, chiefly perivascular in location.   The
middle coat was extensively capillarized, and occasionally a thick-walled arteriole
was observed with marked obliterative hyperplasia of the intima.   The elastic
fibers were distorted, fragmented, twisted and in many areas completely absent.
Muscle fibers had largely disappeared.   There was a sparse number of lymphocytes
and mature fibroblasts, chiefly in the perivascular regions.

The adventitia was markedly thickened by dense fibrous tissue, in which the
vasa vasorum revealed extensive endarterial hyperplasia with considerable nar-
rowing of their lumens.   They were surrounded frequently by slight aggregations
of lymphoid cells.   This process continued unabated into the periadventitial sub-
epicardial tissue.   The Levaditi stain was negative.

Right Aortic Cusp Through the Defective Portion: The media and adven-
titia of the root of the aorta revealed lesions identical with those just described.
The process could be seen extending downward toward the origin of the anterior
flap of the mitral valve.

Superficially (corresponding to the diffuse, pearly, finely striated deposit noted
in the gross specimen) there was a proliferated mass of loose, spongy, cellular
tissue containing many young and mature fibroblasts; a rare thin-walled capillary
was evident in it.

The ridge corresponding to the remnant of the cusp was composed of a tri-
angular, dense, vascularized expansion from the inflamed intervalvular fibrosa. It
contained irregular arrangements of elastic fibers, frequently in compact masses,
occupying chiefly the periphery of the central fibrous area.   Many thin-walled
capillaries and a sparse number of small round cells were present.

Anterior Mitral and Posterior Aortic Cusps: The posterior aortic cusp and
spongy ring were normal.   The annulus fibrosus and the tissue immediately behind
it (fig. 4 A) were moderately invaded by lymphocytes, capillaries and arterioles,
the latter frequently with obliterative endarterial lesions identical with those
described in the aorta.   The process could be traced unchanged in degree or char-
acter through the intervalvular fibrosa and ring of the mitral valve into and along
each side of the fibrous layer of the anterior leaflet (fig. 4 B) for half its length.
Its thickness was thereby roughly quintupled.

The normally delicate auricular layer of elastic fibers became frayed and almost
lost in this region.   Superficial to it was a poorly elastified, well capillarized and
infiltrated fibrous tissue hyperplasia, actually wider than the original fibrosa layer.
On the opposite side a similar but less vascularized proliferation of fibrous tissue
rested on an intact ventricular layer of elastica and was seen to be continuous with
the hyperplastic tissue noted in the region of the defective aortic cusp.

Posterior Cusp of the Mitral Valve: The valve and ring were normal.

Pulmonary Valve: The valve and ring were normal.   The adventitia of the
root of the pulmonary artery presented a picture which was identical with that
in the aorta, and which undoubtedly represented an extension through the subepi-
cardial tissue at the base of the heart.

Tricuspid Valve: The ring and valve were normal.   In the septum fibrosum
the collagen fibers were arranged in whorls.   There was a moderate degree of
capillarization with sparse focal accumulations of lymphocytes.   In the bundle of
His, at this level, could be seen marked intimal proliferation of the arterioles,
occasional lymphocytic aggregations and distinct fibrosis.   The endocardium on
the left aspect of the septum fibrosum was tremendously thickened by a loose,
spongy fibrous tissue proliferation containing extreme numbers of fibroblasts and
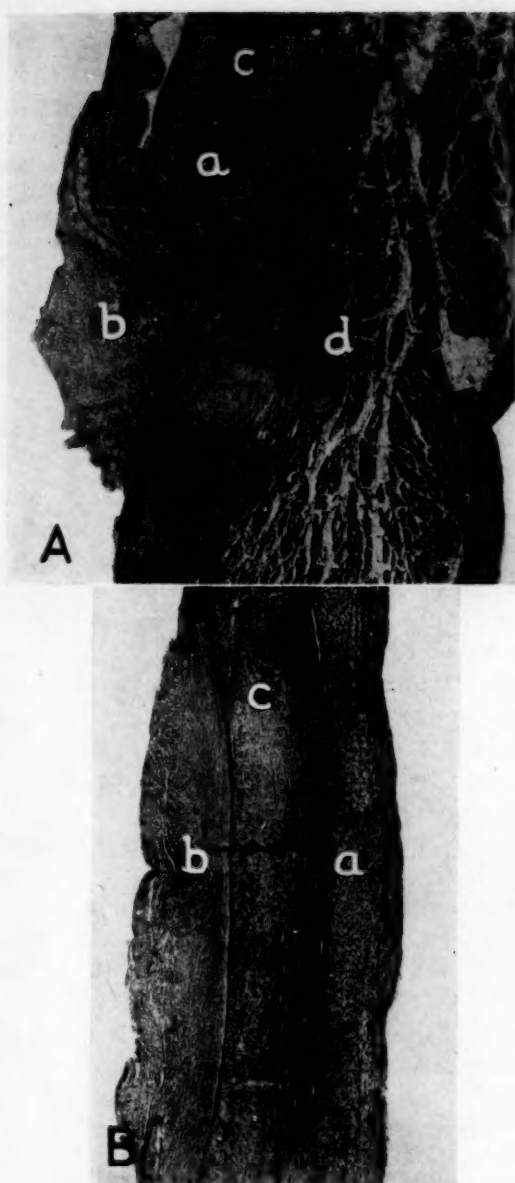occasional capillaries.

Fig. 4 (case 2).—*A*, region of the annulus fibrosus (*a*) of the posterior aortic cusp. Note subaortic proliferation of avascular fibrous tissue (*b*). The inflammatory process in the root of the aorta (*c*) is continuous with that in and behind the annulus. A vessel in the latter situation is obliterated by endarteritic hyperplasia (*d*). *B*, inferior prolongation of the block of tissue from which section *A* was cut. Note the aortic leaflet of the mitral valve, showing broad hyperplastic inflammatory proliferations on the auricular (*a*) and ventricular (*b*) side of the thickened, infiltrated fibrosa layer (*c*). Both *A* and *B* show staining with Weigert's elastica stain, hematoxylin and Van Gieson's stain. Magnification, 35 mm.; × 12.

Left Auricle, Left Ventricle and Right Ventricle: These were normal.

Other Organs: Other postmortem findings of importance were confluent bronchopneumonia of the middle and lower lobes of the right lung, infarction of the lower lobe of the left lung, with acute fibrinous pleuritis, bilateral hydrothorax, hydroperitoneum, edema of the lower extremities, chronic passive congestion of the liver and spleen, old infarct scar of the left kidney and thrombosis of the left subclavian vein near its mouth. No lesions of syphilis were found in any of the other organs.[18]

COMMENT ON CASE 2

The cardiovalvular lesions found in this 20 year old girl were very unusual. The severely scarred aorta, the destructive lesion of the aortic valve and the fibrous thickening of the anterior mitral leaflet were contiguous processes and were unquestionably inflammatory in origin.

The youth of the patient (in whom the clinical history indicated a valvular defect in her eighteenth year) seems to militate against a possible syphilitic etiology and favors the likelihood of rheumatic cardiovalvular disease. However, the latter may be satisfactorily excluded from the diagnosis, since careful study of many sections failed to reveal any lesions suggestive of it.

Microscopic examination of the lesion at the root of the aorta disclosed a very severe grade of aortitis which was recognized by available criteria to be distinctly syphilitic. It is out of all proportion to the rheumatic lesions described in the aorta by Klotz[19] and by Pappenheimer and Von Glahn.[20]

The destructive loss of one half of an aortic cusp suggests the possibility of bacterial endocarditis[21] as the etiologic agent. In the absence of absolute evidence to the contrary, this diagnosis cannot be definitely excluded, although it is hardly likely. In addition, one must not overlook the possible etiologic significance of the paratyphoid and influenzal infections in her childhood.

In searching the literature for a clue as to the nature of this valvular defect, a strikingly similar case was encountered. Spalding and Von Glahn's patient,[22] a man aged 31, with a positive Wassermann reaction of the blood, suffered from cardiac decompensation. Sudden death was found to have been caused by a rupture of the posterior papillary muscle due to an early gummatous focus containing spirochetes. A plaque of supravalvular mesaortitis was found above and involving the left posterior-anterior commissure. The anterior half of the left posterior

18. The brain was not examined.

19. Klotz, O.: Tr. A. Am. Physicians **27**:181, 1912.

20. Pappenheimer, A. M., and Von Glahn, W. C.: Am. J. Path. **3**:583, 1927.

21. The bacterial stain was negative, although this does not exclude a healed stage.

22. Spalding, E. D., and Von Glahn, W. C.: Bull. Johns Hopkins Hosp. **32**:30, 1921.

aortic cusp was entirely destroyed, a small ridge indicating its previous site of attachment. The left half of the anterior cusp was also eroded but to a lesser extent.

The fibrous thickening of the anterior mitral leaflet bore several points of resemblance to the finding in case 1. Reference to the autopsy protocol and to the photomicrographs (fig. 4 *A* and *B*) indicates that here again a similar inflammatory process followed the same path of extension from the base of the aorta to the anterior flap of the mitral valve and on to the endocardium over the membranous septum. However, the mitral valvular and septal lesions were less intense. In fact, the lesion in the bundle of His apparently failed to produce heart block in this case.

Despite the negative Levaditi stains and the age of the patient, the accumulated data point to syphilis as the probable cause of the lesions. The characteristic aortitis, the similarity of the aortic valvular lesion to that in Spalding and Von Glahn's [22] case of proved syphilis and the resemblance of the mitral valvular lesion to that in case 1 and in Friedman's [7] and Staemmler's [8] cases support this view. Unfortunately the patient did not live long enough for a serologic examination; the reliability of the previous examinations is uncertain.

The age of the patient suggests the possibility of congenital tertiary syphilis, which cannot be entirely ruled out. However, the rarity of authentic cases of congenital syphilitic aortitis after infancy [23] and the absence of stigmas of congenital syphilis render such a diagnosis very doubtful.

RECAPITULATION

Acquired tertiary syphilitic heart disease (exclusive of commissural aortitis and the nondiagnostic type of myocardial fibrosis) has heretofore been considered synonymous with gummatous lesions.

However, the two cases reported here presented macroscopic and histologic lesions which, when considered in connection with those in other cases recorded in the literature, are extremely suggestive of syphilis. They point to the existence of tertiary syphilitic cardiac lesions which are not distinctly gummatous. In other words, it appears that, in addition to diffuse and localized gummatous lesions (described in a previous report [1]), a third type of syphilitic involvement can be recognized in which coagulation necrosis and giant cells are absent.

This lesion is apparently very rare. Its gross appearance is that of a fairly well circumscribed, densely sclerotic, whitish, cartilaginous mass. In a valve it produces marked thickening of the structure. The histologic picture is one of dense irregular or whorled connective tissue formation. Valvular elastic lamellae are reduplicated and frayed. The

23. Stolkind, E. J.: Brit. J. Child. Dis. **17**:126, 1920.

entire lesion is apt to be extensively vascularized; capillaries and arterioles with obliterative endarterial lesions are found. These are usually surrounded by focal accumulations of lymphocytes, plasma cells and large mononuclear cells. Diffuse cellular infiltrations are likewise present. In the anterior leaflet of the mitral valve, the process arises by extension from syphilitic disease at the root of the aorta.

In summary, the picture is that of a well scarred granuloma in which necrotic foci have disappeared. The fact that coagulation necrosis is absent should not exclude the diagnosis of syphilis any more than it does in the case of syphilitic aortitis. The lesion in the mitral valve in the cases of Friedman [7] and Staemmler [8] belongs to this type of granulomatous involvement, differing, however, by possessing areas of coagulation necrosis and less prominent fibrosis, i. e., a more active type of granuloma.

SUMMARY

Two extremely unusual instances of cardiovalvular disease are reported in which the accumulated evidence points to a presumptive diagnosis of acquired tertiary syphilitic heart disease.

The lesion encountered represents a well scarred granuloma, most probably syphilitic.

The question is raised as to whether the typical gummatous lesion of the heart (apart from commissural aortic syphilis and the non-diagnostic syphilitic myofibrosis) constitutes the sine qua non of cardiac syphilis.

VASCULAR LESIONS IN SURGICALLY EXCISED STOMACHS

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The frequent observation of vascular lesions in surgically excised stomachs in these laboratories led to the present study of such lesions in relation to gastric and duodenal ulcer. Although much has been written concerning gastric arteriosclerosis, its importance is probably much underestimated. A few workers have held that vascular disease is the primary factor in the causation of peptic ulcer. There are numerous opponents to this view who not only refuse to accept it in its entirety but have set vascular disturbances entirely aside in theoretical considerations of the pathogenesis of peptic ulcer. Admittedly there is no proof that vascular lesions are the main factor in the production of gastric ulcer. However, it seems not unlikely that arteriosclerosis may play a rôle in the localization and persistence of ulcer. With this in mind, a careful, systematic study of the arterial changes in a series of resected gastric specimens was undertaken. Attention was directed particularly to the nature and distribution of the vascular changes. As a result of the study certain conclusions have been drawn in regard to their etiology and their possible significance in the pathogenesis of peptic ulcer.

Arteriosclerosis of abdominal vessels was mentioned by von Rokitansky and Lebert as early as 1852. In 1853 Virchow [1] postulated a relationship between gastric ulcer and arteriosclerosis, but it was not until 1884 that pathologic studies of gastric arteriosclerosis, per se, began to appear in the literature. In that year Gallard [2] published a report on two cases of gastric arteriosclerosis, with autopsy. One was that of a young man, 28 years of age, who had experienced epigastric discomfort and hematemesis. Post mortem there was found a small mucosal erosion, in the base of which lay a tiny artery with aneurysmal dilatation. In the other case, that of a man 51 years of age, autopsy disclosed a similar gastric mucosal erosion, also presenting at its base a small aneurysm of a submucosal artery.

From this time on, case reports of gastric arteriosclerosis cropped up sporadically in the literature. In 1908, Lewin, [3] after reviewing the literature on gastric arteriosclerosis then extant, and adding two cases of his own, concluded: "1. It is possible to have severe arteriosclerosis of the stomach with only slight arterio-

1. Virchow, quoted by Ophüls, W.: Arch. Int. Med. **11**:469, 1913.

2. Gallard, quoted by Hamburger, W. W.: Deutsches Arch. f. klin. Med. **97**:49, 1909.

3. Lewin, A. M.: Arch. f. Verdauungskr. **14**:114, 1908.

sclerosis of the aorta and other vessels. 2. Arteriosclerosis of the stomach may lead to multiple aneurysms. 3. Arteriosclerosis of the stomach may occur in very young people." Buday,[4] in the same year, reported the autopsy in a case of widespread, marked sclerosis of gastric arteries and moderate general arteriosclerosis.

Hamburger [5] in 1909 collected a number of cases with autopsy, beginning with that of Gallard in 1884. He was able to glean twelve case reports from the literature. In four of these an aneurysmal dilatation of a submucosal artery was found in the base of a mucosal erosion. Hamburger noted that in only six of the twelve cases had the tissues been subjected to microscopic investigation, and called attention to the case of Carrier in which no gross lesions could be demonstrated. He noted, moreover, that the ages of the patients ranged from 28 to 79 years, and that in all there was a tendency to general arteriosclerosis.

Hamburger's investigations involved the study of gastric arteries in cadavers of persons of both sexes and of widely varying ages. He compared the arteries of the stomach with arteries elsewhere. The relationship between changes in the arteries and changes in the gastric mucosa was studied. He presented ten cases, five in men and five in women. He found that there were definite changes in the gastric arteries of the six patients who were under 43 years of age. Of the four older patients, two showed no evidence of sclerosis of the gastric arteries, although there was considerable general arteriosclerosis. Of the entire number, six showed slight sclerotic changes within the arteries of the stomach, one presented moderate change and one marked change. Hamburger encountered no aneurysmal dilatations of small submucosal vessels, such as had been described by Gallard, Sachs and Hirschfeld. His cases presented an extreme variability of the arterial lesions in the stomach as to distribution, extent and severity. In three of the cases, the gastric arteries were not involved uniformly, certain branches being affected while others remained completely free. He remarked that there was no rime or reason to the distribution of sclerosis in gastric arteries. Regarding the relationship between general arteriosclerosis and sclerosis of the gastric vessels, Hamburger stated: "From the condition of the larger vessels one cannot come to a conclusion regarding the state of those of the stomach." Finally, he observed that the vessels along the lesser curvature of the gastric wall were most often affected.

Ophüls,[6] in a paper concerned mainly with the consideration of the etiology and pathogenesis of peptic ulcer, tabulated eighteen cases of chronic ulcer of the stomach associated with general arteriosclerosis which had come to autopsy. The gastric arteries were examined in five of the cases; arteriosclerosis was noted either in the arteries in the base of the ulcer or in vessels supplying the region in which the ulcer occurred. In the same communication, he reported four cases of chronic peptic ulcer in young persons. The ages ranged from 24 to 39 years. He was able to find gastric arteriosclerosis, associated with little or no general arteriosclerosis, in each one of this group. Regarding the origin of the local arteriosclerosis, Ophüls stated: ". . . I am inclined to believe that in such instances also one is dealing with a local primary disease of the arteries like that which nobody denies to exist in cases of spontaneous gangrene of the leg in young individuals. The one strong reason in favor of this view is that the disease in the arteries in these cases also usually extends a good distance beyond the base of the ulcer. . . ."

4. Buday, K. V.: Beitr. z. path. Anat. u. z. allg. Path. **44**:327, 1908.

5. Hamburger, W. W.: Deutsches Arch. f. klin. Med. **97**:49, 1909.

6. Ophüls, W.: Arch. Int. Med. **11**:469, 1913.

Zeek and Phair [7] reported three cases that had come to autopsy, in each of which diffuse sclerosis of small vessels was encountered within the gastro-enteric tract. In two of the cases there was massive gangrene of the stomach and intestines. In the third there was extensive ulceration of the enteric tract without involvement of the stomach. Microscopic examination in each case revealed marked intimal thickening of the submucosal arteries, with ulceration and inflammation of the areas of mucosa supplied by them. There was evidence of general arteriosclerosis in all three patients.

From the preceding brief review of the literature it is evident that much serious thought and study have been given to the problem of gastric arteriosclerosis, particularly from a pathologic point of view. A few of the points mentioned, I consider, cannot be overstressed. The suggestion of Ophüls that gastric arteriosclerosis is a local primary disease of the arteries is of importance. It may be seen from the work of both Buday and Hamburger not only that gastric arteriosclerosis is not necessarily associated with general arteriosclerosis, but that general arteriosclerosis, or sclerosis of great vessels, may exist without con-

TABLE 1.—*Conditions Represented in Specimens*

Diagnosis	Number of Cases
Gastric ulcer	7
Duodenal ulcer	13
Secondary (previous operation had been performed)	8
Gastritis (symptoms of ulcer but no ulcer could be demonstrated clinically or pathologically)	2
Total	30

comitant sclerosis of the arteries of the stomach. The finding by Hamburger that the vessels along the lesser curvature of the gastric wall were oftenest affected is of interest. The frequent mention by various writers of involvement of submucosal vessels, particularly in relation to mucosal ulceration, is worthy of note. It seems fairly clear from the descriptions of the nature of the vascular changes that chronic nodular endarteritis was the lesion usually encountered, and that the aneurysmal dilatations of submucosal arteries described by Gallard, Sachs and Hirschfeld fit into this category.

MATERIAL

A series of thirty surgically resected portions of stomach [8] received in this laboratory from April 1933 to April 1934 was studied. Only those specimens removed because of malignant disease were excluded. The patients from whom the specimens were excised ranged from 23 to 63 years of age. The average age was 42 years. The sex ratio was roughly 2:1, as 21 of the specimens were from men and 9 from women. The specimens were grouped as shown in table 1.

7. Zeek, P., and Phair, J. J.: Am. J. M. Sc. **181**:548, 1931.

8. From the Surgical Service of the Toronto General Hospital.

The ulcer was present within the excised specimen in seven of the cases of duodenal ulcer. In the remainder of this group the ulcer was knowingly left behind at operation (Polya operation). Of the eight ulcers listed as secondary, seven had been diagnosed as duodenal ulcer prior to operation or at a previous operation. Each may or may not have been present or active at the time of secondary operation. Stomal ulcers were identified pathologically in the specimens from four of these cases.

The specimens were pinned out on cardboard and fixed in solution of formaldehyde U. S. P. (1:10) as soon as possible following removal, to eliminate the
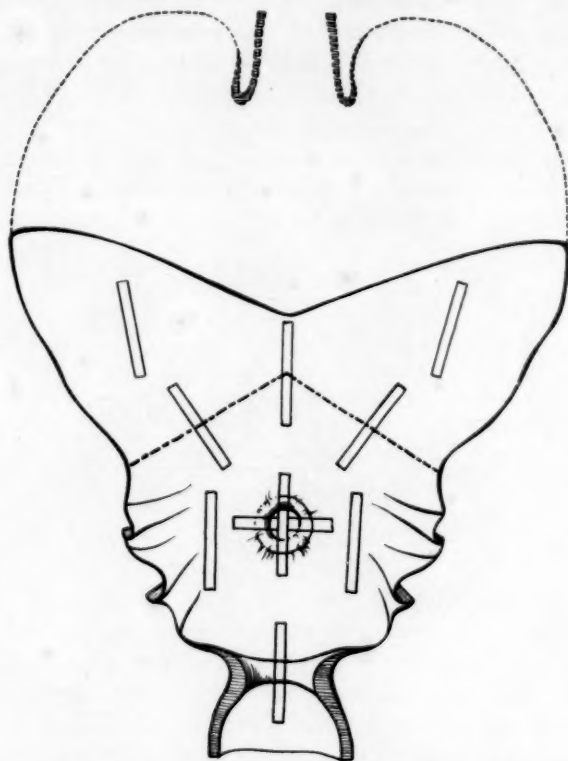
Fig. 1.—A diagram showing the plan followed in the choice of blocks for microscopic examination.

factor of autodigestion. A rough sketch was made of each specimen. Blocks for microscopic study were taken in every case, adhering as closely as possible to the diagram (fig. 1). The site from which each block had been taken was outlined on the drawing of the specimen. Paraffin sections were made and stained with hematoxylin and eosin. In a few instances either elastin H or Weigert's elastic tissue stain was used in addition.

OBSERVATIONS

The results are tabulated. The tables are self-explanatory and deal particularly with the degree of gastritis and the distribution and degree

of arteriosclerosis in each specimen. The cases have been divided into four groups according to the indication for resection. An analysis of each group is given, followed by an analysis in which the cases are divided into two groups according to age. Finally, the nature of the gastritis and endarteritis encountered in the entire series is described.

Analysis of Table 2.—In each of the seven specimens, marked intimal thickening of arteries in the vicinity of the ulcer was noted. Of more interest, however, was the constant occurrence of arteriosclerosis at

TABLE 2.—*Group of Seven Specimens Resected for Gastric Ulcer*

No.	Age	Sex	Description of Specimen	Degree of Gastritis or Duodenitis	Distribution and Degree of Arteriosclerosis
4	45	M	Pylorus with portions of duodenum and fundus; prepyloric gastric ulcer	Marked	Marked intimal thickening of arteries within floor of ulcer; marked in several submucosal arteries some distance from edge of ulcer
6	35	M	Pylorus with portion of fundus; pyloric ulcer, high on lesser curvature	Marked in pylorus, slight in fundus	Marked intimal thickening in vessels in floor of ulcer and in submucosal arteries of pylorus near greater curvature
22	43	M	Pylorus with portions of fundus and duodenum; pyloric ulcer high on lesser curvature	Marked	Marked intimal thickening in floor of ulcer and in number of submucosal arteries in fundus; arteriosclerosis present as distant as 7 cm. from edge of ulcer
24	50	M	Pylorus with portion of fundus and duodenum; small prepyloric gastric ulcer	Marked in pylorus, slight in fundus	Slight to marked intimal thickening in a number of submucosal arteries; lesions not more marked in or near ulcer
25	40	F	Pylorus with portions of fundus and duodenum; penetrating pyloric ulcer high on lesser curvature; hour-glass deformity	Marked in pylorus, moderate in fundus	Marked intimal thickening in base of ulcer and within 2 cm. of edge of ulcer in all directions; marked in a number of small arteries in submucosa, muscularis and subserosa in all blocks
27	46	F	Pylorus with portions of fundus and duodenum; gastric ulcer high on lesser curvature; hour-glass deformity	Marked	Marked intimal thickening within base of ulcer, as well as in a number of submucosal arteries in duodenum, pylorus and fundus, away from ulcer
29	40	M	Pylorus with portions of fundus and duodenum; prepyloric gastric ulcer	Marked in pylorus, slight in fundus	Marked intimal thickening of vessels in and near ulcer; moderate or marked in a few subserosal vessels of fundus; slight in a few submucosal vessels of fundus

some distance from the edge of the ulcer. This was noted particularly in submucosal arteries, whether of the pylorus or of the fundus. The distribution of the arterial lesions was always patchy in that two or three submucosal arteries in a certain block revealed marked intimal thickening, while possibly twenty or more similar vessels in the same block did not present the slightest evidence of pathologic change. The gastritis was marked in the pyloric region in each instance. In four of the seven specimens, however, there was only slight or moderate gastritis in the fundic area. The duodenum, in each instance in which it was present, showed marked duodenitis. The outstanding finding in this

series was, I believe, the occurrence in every case of marked intimal
thickening of a number of submucosal arteries not in the vicinity of
the ulcer.

TABLE 3.—*Group of Thirteen Specimens Resected for Duodenal Ulcer*

No.	Age	Sex	Description of Specimen	Degree of Gastritis or Duodenitis	Distribution and Degree of Arteriosclerosis
1	50	M	Pylorus with portions of duodenum and fundus (ulcer not included)	Moderate	Slight to moderate intimal thickening in several submucosal arteries of pylorus; marked in submucosal arteries of fundus, midway between greater and lesser curvatures
2	56	F	Pylorus with portions of duodenum and fundus; duodenal ulcer	Moderate	Slight intimal thickening in a few submucosal arteries near pylorofundic junction; none in base of ulcer
3	59	M	Cuff of fundus (ulcer not included)	Slight	None
7	49	F	Pylorus with portion of duodenum; duodenal ulcer	Marked	Marked intimal thickening of arteries near and in base of ulcer; also in subserosal and submucosal vessels high in pylorus on posterior wall near lesser curvature
9	32	M	Pylorus with portion of fundus (ulcer not included)	Marked	Slight intimal thickening in a few submucosal arteries, in nearly all of blocks
10	31	M	Pylorus with portion of duodenum (ulcer not included)	Marked	Marked intimal thickening in number of submucosal arteries of duodenum; similar change in only a few arteries of pylorus
11	29	F	Pylorus with portions of duodenum and fundus (ulcer not included)	Moderate	Slight to moderate intimal thickening in a few submucosal arteries in all areas
13	33	M	Pylorus with portion of duodenum; duodenal ulcer	Marked	Marked intimal thickening in arteries at base of ulcer; slight in a few submucosal arteries in other sections
16	58	F	Pylorus with portion of duodenum; superficial duodenal ulcer	Moderate	Marked intimal thickening of submucosal arteries in duodenum at ulcer, and at pyloric ring; moderate intimal thickening of submucosal vessels high in pylorus
20	30	F	Pylorus with portions of duodenum and fundus; duodenal ulcer	Marked in pylorus, slight in fundus	Moderate or marked intimal thickening of submucosal vessels in duodenum and distal portion of pylorus
21	46	M	Pylorus with portion of fundus (ulcer not included)	Marked	Marked intimal thickening in many submucosal branches in pylorus, also in occasional branches in muscularis and subserosa; moderate intimal thickening in submucosal arteries of fundus
26	35	M	Pylorus with portions of fundus and duodenum; duodenal ulcer	Marked	Marked intimal thickening of vessels in vicinity of ulcer; also in submucosal vessels of pylorus and lower portion of fundus
34	38	M	Pylorus with portions of duodenum and fundus; duodenal ulcer	Moderate in pylorus; slight in fundus	Marked intimal thickening of a few tiny vessels in vicinity of ulcer; slight in a few submucosal arteries of pylorus; none in fundus

Analysis of Table 3.—The ulcer was present in seven of the speci-
mens. In six of these there was marked intimal thickening of certain
arteries in the vicinity of the ulcer. No arteriosclerosis was noted,
however, in the base of the ulcer in the remaining one, although there
was slight intimal thickening of a few submucosal arteries high in the
pylorus. In these seven cases, study of arteries in sections away from

the vicinity of the ulcer revealed slight intimal thickening in three, moderate in two and marked in two. In six instances this involvement was of submucosal vessels alone. The other specimen presented marked intimal thickening in both submucosal and subserosal vessels away from the ulcer.

Of the six specimens received in which no ulcer was present, five showed varying degrees of endarteritis and one presented none. Judging from the propinquity of the vascular lesions to the distal edge of the specimen, I believe that in two of the cases the intimal thickening was most marked near the ulcer. In three specimens, however, this did not appear to be the case. Two of these showed slight or moderate intimal thickening of submucosal arteries in all areas. In the third there was marked intimal thickening of several submucosal arteries of the fundus, midway between the greater and the lesser curvature, in addition to marked intimal thickening of arteries in the pylorus.

Of the entire thirteen specimens, gastritis or duodenitis was slight in one, moderate in five and marked in seven. In two instances, the gastritis was less marked in the fundic region. In those specimens which included a portion of duodenum, the degree of duodenitis corresponded fairly well with that of the gastritis. As to the relationship between the gastritis and the endarteritis, little can be said at this point, save that in the specimen which showed only slight gastritis, no arterial lesions were found. In summing up, it seems worthy of note that in this group involvement of submucosal vessels particularly was observed, frequently at some distance from the vicinity of the ulcer. The absence of arterial change in the base of the ulcer in one case is also of interest.

Analysis of Table 4.—Of the eight specimens in this group, four presented stomal ulcers. In two of the latter, intimal thickening of submucosal vessels was more marked in the region of the ulcer. In the other two, arteriosclerosis was no more severe in the vicinity of the ulcer than at a distance. Of the four specimens in which no stomal ulcer was found, one presented no endarteritis. Of the remaining three, two presented marked intimal thickening of submucosal arteries in all areas. The third, in which but a small section of gastric wall was included, presented marked intimal thickening of a few submucosal vessels within its limits.

Gastritis, duodenitis or jejunitis was marked in five cases and moderate in three. I think it worthy of emphasis that in this group, as well as in the two preceding groups, there was evidence of chronic endarteritis distant from any ulcerative process.

Analysis of Table 5.—Although the gastritis was marked in both of these specimens, the endarteritis was of slight degree. The submucosal arteries were involved.

Analysis According to Age.—Sixteen of the patients fell into a group ranging from 20 to 40 years of age. The remainder (fourteen) comprised a group from 41 to 63 years of age. The relative incidence of

TABLE 4.—*Group of Eight Specimens Resected Secondarily*

No.	Age	Sex	Description of Specimen	Degree of Gastritis, Duodenitis or Jejunitis	Distribution and Degree of Arteriosclerosis
5	63	M	Pylorus with portions of duodenum, fundus and jejunum; gastro-enterostomy stoma	Marked	Marked intimal thickening of muscular arteries along greater curvature, near pylorofundic junction; moderate or marked in scattered vessels elsewhere; sclerosis is not more marked in region of stoma
8	23	F	Pylorus with portions of duodenum and fundus; gastro-enterostomy stoma with superficial erosion	Marked	Moderate intimal thickening in one subserous vessel of pylorus on greater curvature; slight intimal thickening of several submucosal branches of pylorus
12	44	M	Pylorus with portions of fundus and jejunum; gastro-enterostomy stoma	Marked	Marked intimal thickening in a number of submucosal arteries in all portions of specimen
14	23	F	Cuff of fundus	Moderate	None
15	34	M	Small ring of gastric wall (fundus), with attached length of jejunum	Moderate	Marked intimal thickening of several submucosal arteries in stomach (fundus)
18	36	M	Pylorus with portion of fundus; gastro-enterostomy stoma; stomal ulcer	Marked	Marked intimal thickening of arteries in vicinity of ulcer; none in remainder of fundus; slight in several submucosal vessels of pylorus
23	·58	M	Pylorus with portions of duodenum, fundus and jejunum; gastro-enterostomy stoma; stomal ulcer	Moderate	Slight to moderate intimal thickening of submucosal muscular and subserosal arteries throughout pylorus and fundus; arteriosclerosis is not more marked in vicinity of ulcer
28	36	F	Pylorus with portions of fundus and jejunum; gastro-enterostomy stoma with superficial erosion	Marked	Marked intimal thickening of submucosal arteries in jejunum, and fundus near stoma; slight to moderate in submucosa of pylorus; moderate to marked in fundus along lesser curvature

TABLE 5.—*Group of Two Specimens Resected for Gastritis*

No.	Age	Sex	Description of Specimen	Degree of Gastritis or Duodenitis	Distribution and Degree of Arteriosclerosis
17	40	M	Pylorus and portion of fundus	Marked	Slight intimal thickening in a number of submucosal arteries in both fundus and pylorus
19	58	M	Pylorus and portion of duodenum	Marked	Slight intimal thickening in a few submucosal arteries near pyloric ring

gastric arteriosclerosis was interesting. Only the arteriosclerosis noted outside of the vicinity of the ulcer was considered in this analysis. Of the first group (from 20 to 40 years), six presented marked arteriosclerosis, three moderate, six slight and one none. Of the second group, eight presented marked arteriosclerosis, three moderate, two slight and one none.

Nature of the Gastritis.—As indicated in the tables, nearly every stomach presented a well marked chronic inflammatory change within its wall. This "chronic follicular gastritis" or "chronic gastritis" was identical with that described by Johnston[9] in these laboratories in an earlier series of cases associated with peptic ulcer. The histologic features of the gastritis in this series can be outlined briefly. The mucous membrane was edematous and heavily infiltrated by polymorphonuclear leukocytes, eosinophils, lymphocytes and plasma cells. Similar cells were often scattered sparsely throughout the submucous and muscular coats. The pylorus presented in many instances a more severe degree
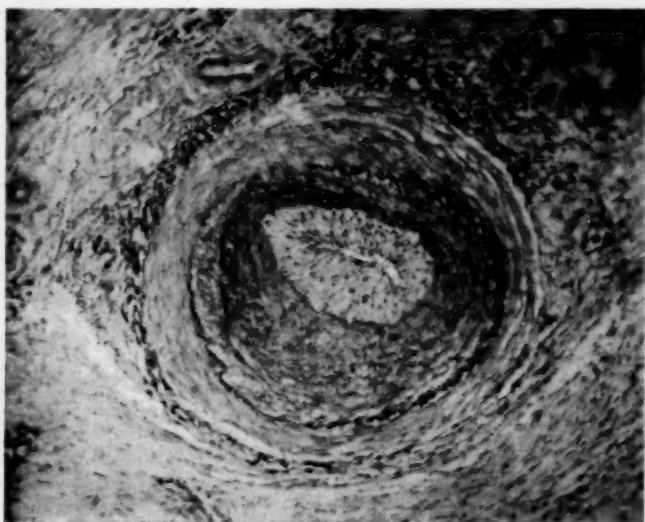

Fig. 2.—A nearly obliterated artery in the floor of a gastric ulcer; elastin H stain. Note the reduplication of the internal elastic lamina, the endothelial proliferation and the inflammatory reaction in the adventitial and perivascular tissue; × 80.

of inflammatory change than the fundus. Hypertrophied lymph follicles were plentiful within the pyloric mucosa of many specimens.

Nature of the Arterial Changes.—The sclerotic changes within the affected arteries were essentially proliferative and involved the intima (chronic nodular endarteritis). Splitting or reduplication of the internal elastic lamina was found in many instances (fig. 2). The submucosal arteries, which were most frequently involved, showed varying degrees of thickening and sometimes marked narrowing of the lumen, even when located far from the vicinity of the ulcer (fig. 3). Although a degen-

9. Johnston, C. R. K.: Surg., Gynec. & Obst. **58**:614, 1934.

erative intimal change had occurred in a few of the involved vessels, it appeared to represent a secondary phase. Medial thickening was not common and when present was slight. No degenerative medial change could be detected. Periarterial or adventitial inflammation was demonstrable within a small number of the affected arteries. This inflammatory reaction was cellular and nonproliferative. The intimal or medial coats were in no instance invaded by inflammatory cells.

COMMENT

A general discussion of the etiology of arteriosclerosis is not within the scope of this communication. Vasomotor or angioneurotic dis-
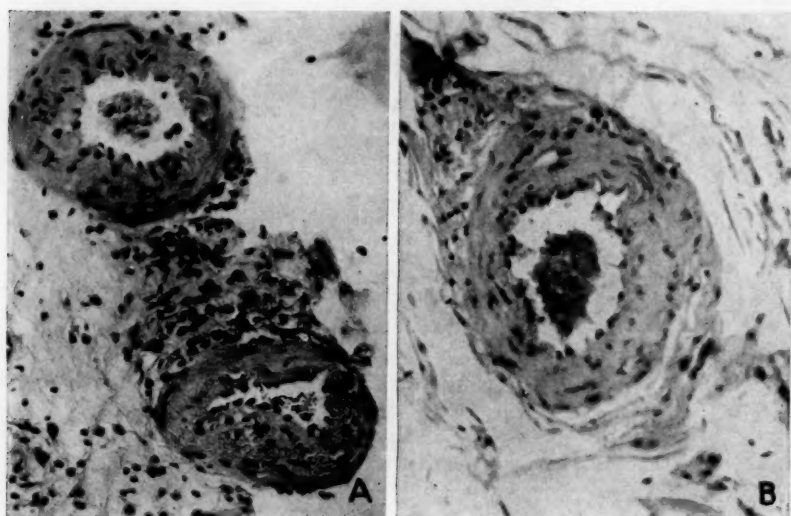

Fig. 3.—*A*, submucosal arteries at the fundus some distance from the ulcer in the same case as figure 2; × 160. *B*, a submucosal pyloric artery some distance from an ulcer, showing slight infiltration by inflammatory cells in the adventitial tissue; × 160.

turbances have been suggested as the cause of arteriosclerosis in general, and of gastric arteriosclerosis in particular (Held [10]). I believe, however, that there is more evidence, experimental and otherwise, that the arteriosclerosis encountered in the present study is of infective origin. The morphologic nature of the arterial changes is entirely compatible with this view. Klotz [11a] showed experimentally that streptococcic or typhoid infections produced intimal proliferation, with splitting of the internal elastic lamina in the ascending aorta and pulmonary artery of

10. Held, I. W.:   M. J. & Rec. **122**:6, 1925.

the rabbit.   Attention has been called to the endarteritis frequently present in arteries situated within infective foci by both Klotz [11] and Andrewes.[12]   The latter pointed out that an artery may become involved either by deposition of bacteria or similar material directly on the intimal coat or by invasion through the vasa vasorum of the adventitia or media. It is not inconceivable that in the series of stomachs under consideration the endarteritis is closely related to the gastritis shown to have been present in all.   The frequent involvement of the submucosal vessels favors this view in that the gastritis is always most marked within the mucosa and submucosa.   The occasional appearance of an active cellular inflammatory reaction about one of the affected vessels lends further strength to the belief.

The tremendous quantity of literature concerning the etiology and pathogenesis of peptic ulcer has been ably summarized by Hurst and Stewart.[13]   Only a few of the present-day theories can be mentioned in this discussion.   Many observers, including Virchow, Ophüls and Hauser, have held that gastric or duodenal ulcers arise as a result of local vascular disturbances.   According to this view, the ulcer is primarily infarctive.   Opponents of this theory apparently do not admit the existence of primary gastric arteriosclerosis.   At present much importance is attached to the experimental work of Mann and Williamson,[14] who were able to produce peptic ulcers in dogs by the institution of surgical duodenal drainage.   Those who prefer the biochemical theory of ulcer causation are afforded a measure of experimental proof by this work.   Lately, the view has been advanced that peptic ulcer is but a phase in the course of gastritis or duodenitis (Konjetzny,[15] Faber [16]).   The almost constant presence of gastritis in cases of peptic ulcer cannot be denied.

The health of any member or physiologic unit of the human body is dependent on the integrity of its circulation.   Functional disturbances or organic lesions may occur if the local blood supply becomes, for any reason, inadequate.   The infliction of slight injuries on poorly vascularized portions of the human integument is frequently followed by the development of chronic persisting ulcers.   Examples are varicose ulcers

11. (a) Klotz, O.: Brit. M. J. 2:1767, 1906; (b) Boston M. & S. J. 156:267, 1907.

12. Andrewes, F. W.: Report on Arterial Degeneration, Rep. M. Off. Local Gov. Bd., London, 1913, Appendix B, no. 1; Second Report on Arterial Degeneration, Rep. M. Off. Local Gov. Bd., London, 1913-1914, Appendix B, no. 1, p. 151.

13. Hurst, A. F., and Stewart, M. J.: Gastric and Duodenal Ulcer, New York, Oxford University Press, 1929.

14. Mann, F. C., and Williamson, C. S.: Ann. Surg. 77:409, 1923. Mann, F. C.: S. Clin. North America 5:753, 1925.

15. Konjetzny, quoted by Faber.[16]

16. Faber, K.: Lancet 2:901, 1927.

and ulcers of poorly nourished skin grafts. The exciting factor of insult or trauma cannot, of course, be discounted. An analogy may exist in the instance of chronic gastric or duodenal ulcer in which highly acid gastric juice serves as the exciting factor, and local lowering of resistance secondary to arteriosclerosis, as the underlying predisposing anatomic factor. Thus, I believe the part played by arteriosclerosis in the pathogenesis of gastric or duodenal ulcer to be contributory or secondary. Even though it is granted that irritative effects of acid gastric juice are most important, the localization of an ulcer to a certain area of the mucous membrane cannot be satisfactorily explained except by the existence of a narrowing of the lumens of certain submucosal vessels. As shown by Reeves,[17] the submucosal arteries of the stomach and duodenum become end-arteries as they enter the mucosa. Each of these terminal vessels supplies an area of mucosa measuring approximately 2.5 mm. in diameter. Reeves found that normally the submucosal arteries along the lesser curvature of the stomach and first inch of the duodenum are more tortuous, narrower and less numerous than elsewhere. Nearly all ulcers arise in one or the other of these locations. Attempts to produce ulcer experimentally by causing embolism or thrombosis of gastric arteries have been successful only when the injected substance became lodged in a submucosal artery (Schridde [18]). The frequent occurrence of mucosal erosions and superficial ulcers in association with gastritis is well known. It seems likely that a mucosal erosion occurring in an area where local resistance has been lowered by vascular disease is more liable to persist and become chronic than one in an area the circulation of which is undisturbed.

CONCLUSIONS

A local arteriosclerosis is commonly present within stomachs resected surgically for gastric, duodenal or stomal ulcer.

The arteriosclerosis is patchy in distribution and affects chiefly the submucosal vessels. It is not necessarily confined to the base or floor of the ulcer, but may be present at some distance from the ulcer.

There is apparently little relation between this local arteriosclerosis and age.

The arteriosclerosis found is essentially a proliferative intimal lesion (chronic nodular endarteritis).

This endarteritis may occur as a part of the gastritis shown to be uniformly present.

It is suggested that gastric arteriosclerosis may, as an underlying anatomic factor, play an important part in the localization and persistence of gastric or duodenal ulcer.

17. Reeves, T. B.: Surg., Gynec. & Obst. **30**:374, 1920.

18. Schridde, quoted by Stewart, M. J.: Brit. M. J. **2**:955, 1923.

EARLY LESIONS FOLLOWING INTRAVENOUS ADMINISTRATION OF A FILTRABLE STAPHYLOCOCCUS TOXIN

A STUDY ON THE DOG AND RABBIT

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Lesions produced by staphylococcus toxin have been observed in laboratory animals since de Christmas [1] first injected toxin into the anterior chamber of the rabbit's eye in 1888. Van de Velde,[2] von Lingelsheim,[3] Mosny and Marcono [4] and Morse [5] were pioneers in this field of investigation. In the early part of the twentieth century Neisser and Levaditi,[6] Neisser and Wechsberg [7] and Neisser and Lipstein [8] made many contributions to the subject. Recently Nicolle and Cesari,[9] Russ,[10] Parker,[11] Burnet,[12] Kellaway and his associates,[13] Gross,[14] Burky,[15] Forssman,[16] Dolman,[17] Borthwick,[18] Rigdon and his asso-

1. de Christmas, J.: Ann. Inst. Pasteur **2**:469, 1888.

2. Van de Velde, H.: Cellule **10**:403, 1894.

3. von Lingelsheim, W.: Aetiologie und Therapie der Staphylokokkeninfectionen, Berlin, Urban & Schwarzenberg, 1900.

4. Mosny and Marcono: Compt. rend. Acad. d. sc. **2**:962, 1894.

5. Morse, J. L.: J. Exper. Med. **1**:613, 1896.

6. Neisser, M., and Levaditi, C.: Action de la toxine staphylococcique sur le rein, Compt. rend. cong. internat. de méd. (Sect. de path. gén. et de path. expér.), 1900, p. 475.

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15. Burky, Earl L.: J. Immunol. **24**:115, 1933.

16. Forssman, J.: Acta path. et microbiol. Scandinav. (supp.) **11**:202, 1932.

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ciates [19] and Von Glahn and Weld [20] have contributed to the study of staphylococcus toxin.

Many investigators consider the lesions in the tissues as due to specific fractions in the staphylococcus toxin. Since no one has made a study of all the lesions in any one animal after the intravenous administration of the toxin it is the purpose of this paper (1) to describe the early lesions occurring in the dog and rabbit and to show that frequently all the lesions occur in the same animal and (2) to discuss the pathogenesis of the lesions.

METHODS AND MATERIALS

Five adult dogs and forty-three adult rabbits were used in this study. The toxin was prepared from a hemolytic strain of Staphylococcus aureus by the method described by Parker, Hopkins and Gunther [21] with few modifications. The dogs were given a single intravenous injection of the toxin, and the rabbits were given one or more intravenous injections at intervals of from thirty minutes to twenty-four hours. A complete autopsy was made usually immediately after death, and the tissues were fixed in Zenker's fluid to which a solution of formaldehyde had been added and in a solution of formaldehyde.

LESIONS IN THE DOG

Blood was present in the abdominal cavity of each of the five dogs. The quantity of blood varied from a few cubic centimeters to 500 cc. No rupture of a blood vessel could be demonstrated. There was no fluid or blood in the pleural, pericardial or cranial cavity. The viscera were always in their normal position.

Hemorrhagic areas were present in the diaphragm of four of the five dogs. They occurred in the muscle and beneath the parietal layer of peritoneum and pleura. The largest amount of blood was always found beneath the pleural surface.

In some of the dogs there was a questionable dilatation of the heart. However, this dilatation was not constant in this group of animals. A varying number of petechiae were found in the epicardium, myocardium or endocardium; frequently petechiae were found in all three locations.

Hemorrhagic areas were present in the pleura and in the parenchymatous tissue of the lungs. In one dog the capillaries were dilated throughout the interstitial tissue. The hemorrhagic areas were located in the alveoli and interstitial tissue and about the blood vessels. There were collections of red blood cells in some of the bronchi.

The spleen was usually purplish brown and moderately swollen. The sinusoids were greatly dilated and filled with red blood cells.

The liver was frequently the same color as the spleen, and when it was sectioned a large amount of blood escaped. In a detailed study of the hepatic lobule the cells were found to be swollen, and often they contained small vacuoles. The sinusoids were dilated and filled with blood. A few necrotic foci were present; these were surrounded by red blood cells, polymorphonuclear leukocytes, mononuclear wandering cells or fragmented hepatic cells. The most conspicuous lesion

19. Rigdon, R. H.; Joyner, A. L., and Ricketts, E. T.: Am. J. Path. **10**:425, 1934.

20. Von Glahn, William C., and Weld, Julia T.: J. Exper. Med. **61**:1, 1935.

21. Parker, Julia T.; Hopkins, J. G., and Gunther, A.: Proc. Soc. Exper. Biol. & Med. **23**:344, 1925.

in the liver was an accumulation of red blood cells about the hepatic arteries and veins. In some instances it was difficult to demonstrate the entire wall of the blood vessels, as the cells in the lumen were continuous with those about the periphery.

In some of the dogs the wall of the gallbladder was edematous and hemorrhagic. The accumulation of red blood cells and the edema were most pronounced in the fibro-elastic tissue in the periphery of the wall. The mucosa of the gallbladder and ducts was grossly normal. There were no stones or obstruction in the biliary passages.

Blood was always present in some portion of the gastro-intestinal tract. The largest quantity was usually found in the ileum. Small hemorrhagic areas were present in the mucosa (fig. 1). In addition, the epithelial cells lining the glands

Fig. 1.—Photograph of hemorrhagic areas in the mucosa of the small and large intestines of a dog that received 10 cc. of staphylococcus toxin intravenously and died one and a half hours later. The hemorrhage is usually restricted to the folds of the mucosa in the colon.

and crypts throughout the gastro-intestinal tract showed all stages of degeneration from simple cloudy swelling to complete degeneration (fig. 2).

The kidneys were normal in size, and the cortical surfaces were smooth. In the animals that died a short time after receiving the toxin the capillaries in the glomerular tufts were dilated and filled with red blood cells. In the dogs that lived for a longer time the endothelial cells and the epithelial cells in the glomeruli and tubules were swollen and granular, and the renal epithelial cells sometimes contained hyaline droplets. Albumin was present in the capsular spaces and in the lumens of the renal tubules in some of the dogs. Essentially every capsular space and the lumen of every tubule was filled with albumin in one dog that lived for six hours after receiving the toxin. In this animal the glomerular tufts

appeared to be compressed by the albumin in the capsular spaces. The capillaries in the interstitial tissue were dilated and filled with red blood cells, although similar cells were rarely found in the tubules. In all the dogs the lesions in the kidneys were diffuse and bilateral. There were no lesions of interest in the genito-urinary tract except those in the kidney.

There were no lesions in the pancreas or in the salivary glands in the one dog in which these glands were examined.
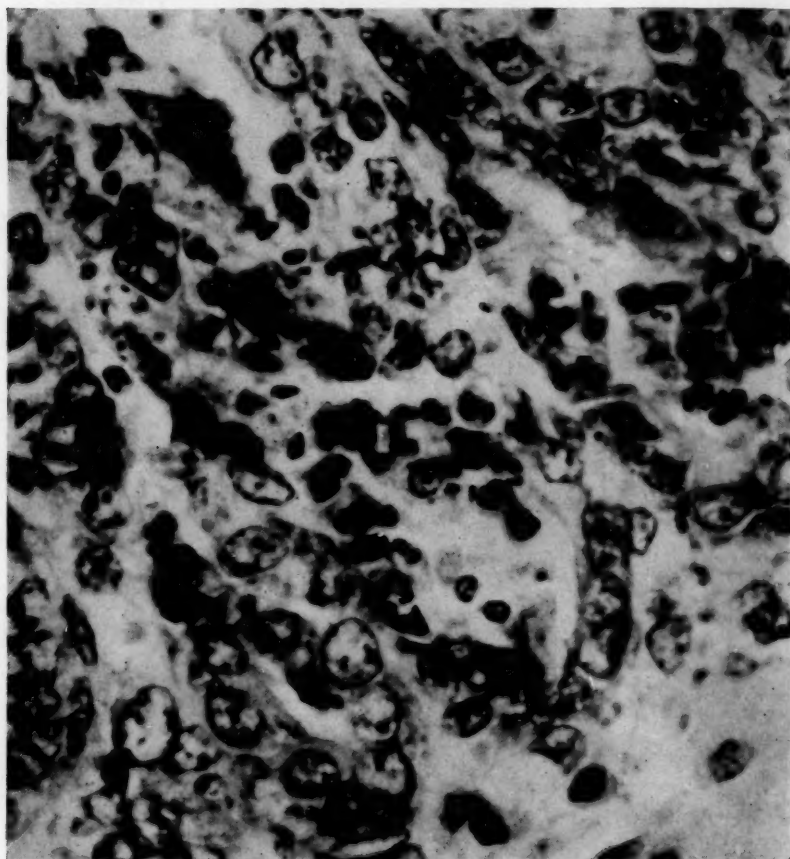
Fig. 2.—Photomicrograph of the basal portion of the mucosa of the ileum of a dog that received 10 cc. of staphylococcus toxin and died six and three-quarters hours later. Note the extensive degeneration of the epithelial cells lining the glands and the accumulation of these cells in the lumens. Similar changes were present throughout the mucosa of the gastro-intestinal tract.

The mesenteric and retroperitoneal lymph nodes were swollen and reddish gray; the sinuses were dilated and filled with red blood cells and phagocytic cells in the dogs in which a large quantity of blood was found in the abdominal cavity.

The only lesions found in the brain were small hemorrhagic areas which were most frequently located about blood vessels.

LESIONS IN THE RABBIT

There were no lesions of interest in the serous cavities.

Petechiae were present in the myocardium, endocardium or epicardium, often in all three locations. Few areas of Zenker degeneration were present in the cardiac muscle.

Small hemorrhagic areas were located in the pleura and in the parenchymatous tissue of the lungs. The capillaries in the interstitial tissue were dilated and filled with red blood cells; similar cells were found in groups of the alveoli and in some of the bronchi.

In some of the rabbits the spleen was purplish brown and moderately swollen. The sinuses were filled with red blood cells, polymorphonuclear leukocytes and phagocytic mononuclear cells. Hemosiderin was free in the sinuses and in phagocytic cells.

The liver was purplish brown; when it was sectioned a large amount of blood escaped. In some rabbits small hemorrhagic areas were found in the liver; these had no definite relation to the hepatic lobules or to the blood vessels. The hepatic
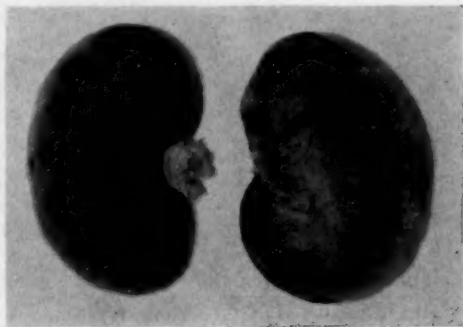

Fig. 3.—Diffuse necrotic and hemorrhagic foci in the cortex of the kidney of a rabbit that received 0.5 cc. of staphylococcus toxin intravenously and twenty-two hours later received an additional dose of 0.75 cc. of toxin. The animal died twenty-three and a half hours after receiving the second injection.

cells were compressed about the areas of hemorrhage. A few foci of necrosis were found which were not associated with any evidence of hemorrhage. In some instances polymorphonuclear leukocytes surrounded the necrotic areas.

In one rabbit there was a localized area of hemorrhage and necrosis from 1 to 2 cm. in diameter in the mucosa of the cardiac portion of the stomach. The only lesions in the small intestine were a few petechiae in Peyer's patches. The most interesting lesion in the gastro-intestinal tract was found in the first 40 cm. of the colon. The wall was often hemorrhagic and edematous, and the mucosa was hemorrhagic or covered with a diphtheritic membrane. In the rabbits in which the hemorrhagic areas were less diffuse the epithelial cells lining the glands showed varying degrees of degeneration. Many of the epithelial cells were completely destroyed, and the fragments filled the lumens of the glands. A few polymorphonuclear leukocytes and mononuclear wandering cells were present in the mucosa of the colon. Circumscribed pink areas, which suggested agglutinated red blood cells or thrombi, filled a few of the capillaries in the mucosa.

The quantity of marrow in the femur varied in the different rabbits. In some rabbits the marrow appeared to be hyperplastic. No lesions were found in either the myeloblastic or the erythroblastic tissue.

There were no lesions in the pancreas or adrenal glands.

The lesions most constantly observed in this series of rabbits were in the kidney. The renal lesions were characterized by cortical necroses and evidence of hemorrhages (fig. 3). The necrosis was either focal or diffuse and was always limited to the cortex. The kidneys were swollen and hyperemic in the rabbits that died from two to ten hours after receiving the staphylococcus toxin. The capillaries in the glomerular tufts were dilated and filled with red blood cells, and the tufts almost completely filled the glomerular space. Albumin was sometimes present in the capsular space and in the tubules. In many rabbits the tubular epithelial cells were often swollen and contained hyaline droplets; this was observed especially in the animals that lived for twelve hours or longer after receiving the toxin. Only a few red blood cells were present in the capsular spaces and in the collecting tubules of the kidney. There was a layer of polymorphonuclear leukocytes and phagocytic cells at the margin of the necrotic zone.

COMMENT

The existence of such fractions as nephrotoxin, leukocidin, hemolysin and skin-necrotizing and acute killing fractions in staphylococcus toxin is not discussed here.[22]

The potency of staphylococcus toxin is influenced by the organism, mediums and method of preparation.[23] The dog and rabbit, two of the species of animals frequently used in experimental work, have been used in studying the action of staphylococcus toxin. Death occurs in both when a sufficient quantity of a potent toxin is given. The lesions are influenced by the quantity of toxin, the methods of administration and the length of time elapsing before death. The lesions may conveniently be divided into two groups, namely, hemorrhagic and necrotic; in this study the former were more frequently encountered in the dog and the latter, in the rabbit.

Swollen and necrotic endothelial cells are found in the capillaries of the rabbits which survive for twenty-four hours or longer. Collections of red blood cells are present about the periphery of many of the blood vessels; this accounts for the small hemorrhagic areas seen in the viscera of the dogs and rabbits and also for the blood observed in the abdominal cavity of the dogs.

The hemorrhagic and necrotic areas suggest that the toxin either injures the endothelium of the capillaries, causing thrombosis and infarction, or injures the epithelial cells by acting directly on those cells. Although there are thrombi in some of the capillaries in the mucosa of the intestinal tract, there is apparently no direct anatomic relation

22. Weld, Julia T.; Parker, and Gunther, Anne: J. Exper. Med. **54**:315, 1931. Burnet.[12]

23. Burnet, F. M.: J. Path. & Bact. **33**:1, 1930. Parker.[11]

between the thrombi and the areas of necrosis. Furthermore, the number of thrombi is too small to produce the extensive necrosis found in some of the animals. The thrombi resemble agglutinated red blood cells more than the thrombi formed by leukocytes and fibrin. The presence of areas of hemorrhage and necrosis in the mucosa of the stomach and of the small and large intestine suggests that the toxin is excreted into any part of the gastro-intestinal tract and that the lesions are produced at the point of excretion rather than at the point of accumulation of the toxin. One of the first changes in the intestinal tract is swelling and degeneration of the epithelial cells lining the glands in the deeper portion of the mucosa. If the necrosis of the mucosa is the result of the action of the toxin accumulated in the colon, the superficial portion of the mucosa should show the first and most extensive necrosis.

The intestinal mucosa is one of the routes of excretion of toxic materials. For instance, aluminum when given intravenously is excreted by the mucosa of the stomach and of the small and large intestines.[24] Likewise, mercurochrome is quickly excreted into the gastro-intestinal tract of the dog after intravenous administration, as shown by the emesis of intensely red material;[25] also, necrosis in the colon of rats following the intravenous injection of mercurochrome has been described by Baldwin.[26]

The intestinal lesions in the dogs and rabbits after intravenous injection of staphylococcus toxin are similar to those found in guinea-pigs after inhalation of mercurial preparations. In a study of the tissues of guinea-pigs which had been given inhalations of mercurial preparations Gutman[27] found evidence of necrosis in the epithelial cells of the villi and of ulceration and shredding of the mucosa in the gastro-intestinal tract. The blood vessels were congested and distended with agglutinated red blood cells, and extravasations of blood were frequently observed. The changes in the large intestine resembled those in the small; however, the ulcerative process was greater in the latter.

While the lesions in the kidney at the time of death differ in the dog and in the rabbit, similar changes are present at times in both species of animals. The absence of cortical necrosis in the dog's kidney can be explained on the basis that death intervenes before sufficient time elapses for the necrosis to occur. Neisser and Wechsberg[7] con-

24. Underhill, F. P.; Peterman, F. I., and Steel, S. L.: Am. J. Physiol. **90**:52, 1929.

25. Bargen, J. A.; Osterberg, A. E., and Mann, F. C.: Am. J. Physiol. **89**: 640, 1929.

26. Baldwin, W. M.: Proc. Soc. Exper. Biol. & Med. **25**:679, 1927.

27. Gutman, J.: Am. J. Syph. **7**:1, 1923.

sidered the cortical necrosis to be the result of thrombi which were formed by the disintegration of leukocytes. Rigdon and his associates [19] were unable to demonstrate a sufficient number of thrombi to consider the necrosis secondary to obstruction and expressed the opinion that the lesions in the kidney were the result of the action of the toxin on the renal epithelial cells and on the endothelial cells of the capillaries. This explanation apparently is the more plausible one.

It is not surprising that débris, phagocytes and hemosiderin are found in the spleen, for Neisser and Wechsberg [7] have shown that red blood cells are hemolyzed by staphylococcus toxin, and Van de Velde [2] has shown that leukocytes are destroyed by it. The presence of a large amount of hemosiderin in the spleen of rabbits dying five minutes after receiving a lethal dose of the toxin suggests that some hemosiderin is present in the spleen of normal rabbits.

In considering the pathogenesis of the lesions in the dog and rabbit two types of lesions must be thought of, namely hemorrhagic and necrotic. The distribution of the former appears to indicate an involvement of the circulatory apparatus, while the latter are usually found in the tissues which have an excretory function. Although there is some evidence in favor of the idea that the necrosis is the result of vascular obstruction, that evidence is not at all conclusive. The two types of lesions may be adequately explained on the basis of direct injury to any cell with which the toxin comes in contact. The endothelial cells of the capillaries are injured, and as a result the permeability of the wall is increased, permitting the escape of red blood cells.

SUMMARY

Hemorrhage and necrosis are the characteristic lesions found in the dog and rabbit after the intravenous administration of staphylococcus toxin. The type of lesion is influenced by the quantity of toxin and the length of time elapsing between the time of injection and the time of death. The areas of necrosis are most frequently found in the kidneys and in the colon of the rabbit while the hemorrhagic areas usually occur in the gastro-intestinal tract and peritoneal cavity of the dog. It appears that the hemorrhages are the result of the action of the toxin directly on the endothelium of the capillaries and small blood vessels, permitting the escape of red blood cells, and that the necrosis is the result of the action of the toxin directly on the cells of the body.

HISTAMINE AND LEUKOCYTOSIS

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AND

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PHILADELPHIA

The presence of histamine in various mammalian tissues has led to intensive investigations to determine its physiologic significance. Some of these [1] indicate that one of its functions has to do with initiating the vascular phases of inflammation following injury to tissue. Pertinent to this is the question what relationship, if any, histamine may have to the leukocytic phenomena associated with inflammation. Experiments have failed to demonstrate that histamine phosphate has any chemotactic effect on leukocytes. The evidence concerning the influence of histamine on the leukocytes in the blood is inconclusive. Some have noted leukopenia, and some leukocytosis, following injections of histamine. Several of the reports are based on incidental observations in which the evidence is fragmentary. There is closer agreement between the reports in which the effect of histamine on leukocytosis was the chief object of the experiment.

Port and Brunow [2] made an incidental observation on leukocytosis following injections of histamine into a dog. The dog weighed 4.4 Kg. and received three injections of 2.2 mg., 3.3 mg. and 1.1 mg., respectively, at intervals of several days. Leukocytosis followed each injection and was most marked following the last injection. The authors believed that the previous injections had made the animal more sensitive to the effects of histamine.

Dale and Laidlaw [3] reported marked leukopenia accompanying shock due to histamine in cats. The observations were made incidental to studies on the nature and mechanism of shock and on the accompanying changes in blood volume and blood concentration. The leukocytic phenomena were not primary objects of the experiments, and no data showing the number or the intervals of time at which the counts were made

1. (a) Lewis, Thomas: The Blood Vessels of the Human Skin and Their Responses, London, Shaw & Sons, 1927. (b) Krogh, A.: The Anatomy and Physiology of the Capillaries, New Haven, Yale University Press, 1929.

2. Port, F., and Brunow: Arch. f. exper. Path. u. Pharmakol. **76**:239, 1914.

3. Dale, H. H., and Laidlaw, P. P.: J. Physiol. **52**:355, 1919.

were recorded. Cats, under ether anesthesia, received histamine in
amounts sufficient to produce death or profound shock promptly. The
recorded intervals between the injections of histamine and death ranged
from fourteen to thirty-four minutes. The observations must have been
made during this period and were obviously intended by the authors to
apply only to animals in profound shock immediately following large
doses of histamine. The assumption that leukopenia would result at
longer intervals following smaller doses of histamine is not warranted
by these observations.

Flatow and Hüttel [4] observed the effects of subcutaneous injection
of histamine in cats, guinea-pigs, rabbits and dogs. Except in rabbits,
this was followed by marked leukocytosis in each case. The blood pic-
ture following subcutaneous doses sufficient to produce shock resembled
that of severe acute infection. On subsequent days there was an increase
in immature forms—"a shift to the left"—followed by an increased
number of monocytes and finally by eosinophilia. A transient leukopenia
followed shock doses of histamine in rabbits. Paul [5] found no significant
changes in the leukocytic count in rabbits following injections of
histamine.

Most of the observations made on human subjects were incidental
to the use of histamine for therapeutic purposes, and in most instances
the data are fragmentary. Berri and Weinberg [4] and Morreti [4] reported
leukopenia following histamine. Motta [6] injected 1 mg. of histamine
into the gluteal muscles of five pregnant women. Leukocytic counts
were made five, fifteen and thirty minutes following each injection. The
results varied. Some counts were slightly higher and some slightly
lower than normal, and some showed no significant variation. No sub-
sequent counts were recorded. Andreoli and Lucchi [7] recorded a mod-
erate leukocytosis in a small group of cases following the therapeutic
use of histamine. Weiss, Robb and Ellis [8] found leukocytosis, the
degree of which was not stated, following slow intravenous instillation
of histamine for therapeutic purposes in five cases.

We have failed to find other observations on a relationship between
histamine and leukocytosis either in animals or in man. Since the avail-
able evidence is inconclusive, experiments were undertaken to secure
additional data. The leukocytes in the blood of adult cats were counted
before and at intervals following the intravenous injection of small

4. Quoted by Feldberg, W., and Schilf, E.: Histamin, seine Pharmakologie
und Bedeutung für die Humoralphysiologie, Berlin, Julius Springer, 1930.

5. Paul, J. R.: Bull. Johns Hopkins Hosp. **32**:20, 1921.

6. Motta, G.: Arch. d. ostet. e ginec. **16**:66, 1929.

7. Andreoli, G., and Lucchi, G.: Minerva med. **7**:1117, 1927.

8. Weiss, S.; Robb, G. P., and Ellis, L. B.: Arch. Int. Med. **49**:360, 1932.

amounts of a sterile solution of histamine phosphate. No anesthesia was used; hence the results may be regarded as uncomplicated by the action of drugs incidental to experimentation. The doses of histamine and the leukocytic counts are presented in table 1.

Differential counts showed that the increase consisted of polymorphonuclear neutrophils. In each instance the blood picture returned to normal within twenty-four hours. In three instances (1, 2 and 4) a decrease in the number of leukocytes was seen within one hour. If subsequent counts had not been made, these results would have indicated that leukopenia follows injections of histamine. But in each instance the leukopenia was transient and was followed by definite leukocytosis.

TABLE 1.—*Leukocytic Counts Following Intravenous Injections of Histamine Into Cats*

Experi-ment	Count Before	Hist-amine, Mg.	Counts After Given Intervals					
			½ Hour	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
1	12,600	2.0	11,800	9,400	16,100	23,700	......	19,250
2	12,750	1.0	8,800	......	25,600	22,400	20,350	......
3	13,850	2.0	......	26,400	52,650	38,200	34,900	...,...
4	13,900	2.0	......	6,200	28,900	32,800	19,000	......
5	13,500	1.0	......	13,700	......	14,950	......	24,250
6	10,350	1.0	......	13,400	25,500	......	......	......
7	15,200	2.0	......	17,800	25,800	24,100	......	......
8	12,400	1.5	......	31,150	34,750	22,000	......	......
9	11,300	2.0	13,800	17,900	24,600	36,000	28,900	......
10	13,500	1.5	14,350	16,800	17,350	17,800	33,700	34,300
11	16,300	2.0	20,500	24,500	......	36,600	......	28,500
12	13,000	1.5	......	18,700	......	21,500	......	29,100
13	14,200	2.0	......	20,000	......	......	......	20,800
14	15,600	2.0	......	21,000	......	22,250	......	30,600
15	9,200	2.0	10,700	10,250	......	24,450	......	39,900
16	16,000	2.0	20,500	25,300	32,600	......	20,600	......
Average	13,350		14,350	18,160	27,380	25,900	26,240	28,340

Injections of 2 mg. usually produced transient shocklike phenomena. Within one or two hours the behavior and appearance of these cats were not distinguishable from those of cats that had not been treated. Eight cats were used in this experiment, and each one was given injections of corresponding volumes of physiologic solution of sodium chloride for control observations. In no instance was there a significant variation in the leukocyte count following injections of saline solution. In our hands the intravenous injection of histamine produced leukocytosis regularly in cats.

In another experiment monkeys (Macacus rhesus) were given subcutaneous injections of varying doses of histamine phosphate in physiologic solution of sodium chloride. Leukocyte counts were made immediately before and at intervals following the injections. Aseptic technic was maintained. Three monkeys were used, and no subsequent

injection was made until the leukocytosis following the previous injection had subsided.   Usually three or four days intervened between injections.   The pulse and respirations at frequent intervals following the injection of histamine showed slight variations from normal.   The manifestations of circulatory disturbances resembling shock were not marked.   The doses of histamine and the leukocyte counts are given in table 2.

It will be seen that a sharp·rise in the leukocyte count followed the injection of even 0.75 mg. of histamine.   The increase consisted of polymorphonuclear neutrophils.   The maximum leukocytosis usually occurred from two to four hours following the injection.   However, in one instance, when 9 mg. of histamine phosphate was injected, the maximum leukocytosis, 57,550, occurred within an hour.   This result may be compared to the high leukocytosis which follows extensive burns of

TABLE 2.—*Leukocytic Counts Following Subcutaneous Injection of Histamine Into Monkeys*

Count Before	Hist- amine, Mg.	Counts After Given Intervals					
		1 Hour	2 Hours	4 Hours	6 Hours	24 Hours	48 Hours
5,550	9.0	57,550	36,800	26,700	20,750	18,950	11,100
6,650	6.0	14,400	16,250	41,400	36,000	17,000	17,150
5,450	4.5	24,150	19,400	7,850	7,780	4,400	9,100
12,050	2.5	25,750	26,100	23,100	24,700	12,300	7,150
7,700	2.25	9,050	22,850	15,000	16,300	......	12,350
5,430	1.5	20,000	40,000	19,400	18,600	14,300	11,000
6,500	1.0	6,150	13,650	16,650	15,700	11,200	8,000
5,400	0.75	15,450	18,850	18,940	19,650	9,800	9,200

the skin in man.   In our hands the subcutaneous injection of histamine was followed by leukocytosis in monkeys.

Histamine phosphate in doses of from 0.5 to 1 mg. in sterile solution was given intravenously to seven young men.   The leukocytes in the blood were counted immediately before and at intervals following the injections.   A moderate leukocytosis occurred regularly, with an average increase of 3,000 leukocytes from three to five hours following the injection.   The count returned to normal within twenty-four hours. In several instances a slight leukopenia was found one hour after the injection.   This was followed by an increase in the leukocyte count in each case.   Control counts at corresponding intervals were made on the same subjects.   In no instance did these show a significant variation. Data concerning this group are shown in table 3.

The intravenous injection of histamine produced a characteristic circulatory reaction.   The face at first was flushed but immediately became pale.   This was accompanied by a metallic taste in the mouth, dizziness, faintness and frontal headache.   The radial pulse was rapid

and sometimes weak. The headache sometimes lasted several hours, but the other manifestations disappeared within thirty minutes. In one instance, case 3, the subject fainted when only 0.5 mg. had been injected. One hour later his leukocytic count had decreased from 5,900 to 1,700. The count rose immediately to 9,800, and twenty-four hours later it was 9,000. This was the only instance in which the leukocytosis in our subjects persisted twenty-four hours.

TABLE 3.—*Leukocytic Counts Following Intravenous Injection of Histamine into Human Subjects*

Subject	Normal Count	Histamine, Mg.	Counts After Given Intervals		
			1 Hour	3 Hours	5 Hours
1	5,000	0.75	9,050	9,700	8,750
2	7,900	0.75	8,350	9,300	9,150
3	5,900	0.50	1,700	9,800	8,750
4	6,350	1.00	6,250	9,450	6,600
5	8,000	0.75	7,650	8,400	15,000
6	6,500	1.0	5,800	4,350	8,200
7	7,800	1.0	5,350	13,600	14,100

TABLE 4.—*Leukocytic Counts Following Subcutaneous Injection of Histamine Into Human Subjects*

Subject	Count Before	Hist- amine, Mg.	Counts After Given Intervals				
			1 Hour	3 Hours	5 Hours	7 Hours	24 Hours
1	5,700	2.0	7,650	6,600	7,700	.....	.....
2	6,240	2.0	6,000	7,150	5,300	.....	6,200
3	7,100	2.0	7,000	6,500	7,200	.....	7,150
4	4,800	2.5	5,650	9,450	6,000	.....	5,000
5	6,500	3.0	6,950	7,200	8,700	.....	6,050
6	6,750	3.0	5,250	5,000	7,200	.....	5,650
7	5,150	5.0	3,800	6,800	7,600	7,600	5,000
8	8,500	5.0	6,500	10,450	11,300	10,600	8,600
9	5,350	5.0	4,400	8,600	8,650	9,600	5,100
10	6,400	5.0	5,300	9,700	10,150	8,700	6,200

Histamine phosphate was given by subcutaneous injection to another group of volunteers. In these the results varied with the amounts of histamine given. In three subjects receiving 2 mg. the leukocyte counts remained within normal limits throughout the course of the experiment. Subject 4, receiving 2.5 mg., showed a maximum rise of 4,650 leukocytes in three hours. Subject 5, receiving 3 mg., showed a maximum increase of 2,200 in five hours, but subject 6, receiving the same dosage, showed little or no effect. In the four subjects given 5 mg. of histamine phosphate a decrease in the number of leukocytes occurred in the first hour followed regularly by an average increase of 3,340 leuko-

cytes in from five to seven hours. Control counts made at correspond-
ing intervals on another day showed no significant variations from
normal. The polymorphonuclear neutrophils were the only cells show-
ing significant numerical variations both in this group and in those
receiving histamine intravenously.

The subcutaneous injections produced general manifestations similar
to those following intravenous injections, but less in degree. Rapid
pulse and flushing of the face were noted within a few minutes. A few
of the men had headache, which lasted several hours; some noticed
shortness of breath on exertion; in others these effects were not present.
The local effects were less marked than had been expected. The injec-
tions were made into the loose areolar tissue near the elbow. They
resulted in slight local swelling and tenderness to pressure for from
twenty-four to forty-eight hours. In no case did this cause complaint,
and it was regarded as trivial by each of the subjects.

COMMENT

It has been shown (Lewis [1a]) that any type of injury to the human
skin results in a circulatory reaction which is local in origin and is
independent of innervation. This reaction depends on the release of
a diffusible substance from the injured cells, which substance is respon-
sible for initiating the early phases of inflammation about the point of
injury. Lewis could not distinguish this substance from histamine but,
lacking evidence concerning its exact chemical nature or combination,
he speaks of it as H-substance. Dale [9] confirmed the conclusions of
Lewis, and stated that there is as good chemical evidence of the pres-
ence of histamine in the cells of the body generally, and of its liberation
from them following injury, as of the existence of epinephrine in the
adrenal medulla, and of its secretion as such into the blood.

Lewis attributed the systemic phenomena which follow extensive
superficial injuries, such as burns, to the liberation of large amounts
of H-substance from the injured skin. It is well established that shock,
similar in all respects to that resulting from injection of histamine,
follows extensive superficial burns. It also is well known that such
burns are followed immediately by marked leukocytosis. Locke [10]
reported counts ranging from 10,000 to 50,000 within two and one-half
hours following burns. The leukocytic counts were above 50,000 regu-
larly in the fatal cases. This raises the question whether such leuko-
cytosis is due to H-substance or to some other agent, and whether
H-substance is identical with histamine in its influence on leukocytes.

9. Dale, H. H.:   Lancet **1**:1235, 1929.
10. Locke, E. A.:   Boston M. & S. J. **147**:480, 1902.

Physiologic assays (Lewis [1a]) indicate that histamine, estimated in terms of the base, is present in the human skin in a concentration of about 1 : 60,000. A simple calculation shows that 5 mg. of histamine phosphate, which contains approximately 2.5 mg. of the base, will be contained in about 150 Gm. of skin. In several of our human subjects the subcutaneous injection of 5 mg. of histamine phosphate was followed by a leukocytosis of 10,000. This approximates relatively, though perhaps it does not equal, the leukocytosis which follows moderately extensive burns of the skin. However, the experimental leukocytosis is very similar in degree to that which regularly follows extensive surgical procedures when no infection is present. Postoperative leukocytosis may be due in part to the absorption of histamine from areas of traumatized tissue.

It must be remembered that the chemical combination in which histamine exists in tissues is not known. It is possible that that combination is more effective in its physiologic action than is histamine phosphate.

Higher leukocytic counts were obtained in cats and in monkeys than in man. This probably was due to the larger dose relative to the body weight.

We express our appreciation to the group of medical students who voluntarily submitted to experimentation in the work here reported.

CONCLUSIONS

Histamine phosphate given intravenously to cats, subcutaneously to monkeys and by both methods to man is followed by an increase in the number of polymorphonuclear leukocytes in the blood.

Frequently the leukocytosis following injections of histamine is preceded by a transient leukopenia.

The release of histamine from cells in areas of extensive injury is probably a factor in evoking the subsequent leukocytosis. This may account for the leukocytosis following surgical and other injuries.

BEHAVIOR OF TRANSPLANTED SPLEEN

WITH SPECIAL REFERENCE TO THE TISSUE DIFFERENTIAL OF HEMOPOIETIC ORGANS

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Various hemopoietic organs can be transplanted, but with much less success than epithelial tissues. I wished to analyze this deficiency in transplantability, and for this purpose I studied transplantation of splenic tissue in guinea-pigs. In particular it seemed of interest to determine, if possible, (1) how regeneration of splenic tissue takes place under the conditions of transplantation, and (2) why hemopoietic tissues show only very slight regenerative and growth tendency.

MATERIAL AND METHODS

Autotransplantations, syngenesiotransplantations and homeotransplantations of splenic tissue were carried out in guinea-pigs weighing from 200 to 250 Gm. Pieces of spleen, about 4 by 4 mm., were transplanted immediately after removal into subcutaneous pockets of the abdominal wall. The grafts were taken out together with the surrounding tissue and cut in serial sections. Twenty-four animals were used in these experiments.

OBSERVATIONS

Autotransplantation.—After two days the peripheral zone of the graft is alive; the reticulum cells are producing free wandering phagocytotic histiocytes. Living malpighian bodies are also seen, but show no signs of regeneration; a slight invasion of the transplant by some lymphocytes from the blood stream of the host is noted. The central zone of the transplant has become necrotic in some places. There are here no invading leukocytes and lymphocytes; no mobilization of reticulum and endothelial cells is noticeable, nor is there any marked activity of the malpighian bodies. The power of resistance of the latter is striking. A certain number of the lymphocytes in these structures are alive, their cytoplasm somewhat increased, but other lymphocytes are damaged or destroyed. An augmentation of mitotic figures is not observed. Four days after transplantation the inflammatory cells in the peripheral zone have disappeared. Here the reticulum cells have become mobilized and transformed into histiocytes, which are numerous and are active phagocytes. Many lymphocytes of the

malpighian bodies show an increase in cytoplasm, and some of these cells also have become free wandering cells, having thus been changed into polyblasts. The central zone of the graft shows signs of injury, some lymphocytes being destroyed. However, here also a large number of lymphocytes of the malpighian bodies are well preserved, and they likewise become converted into polyblasts which penetrate through the necrotic zone and reach the living tissue in the periphery. The polyblasts thus have two points of origin: Some arise from the peripheral living zone of the lymphoid tissue as well as from the transformed reticulum cells in this region, while others develop from the surviving cells in the central zone. Mitotic figures are seen everywhere in the lymphocytes, but their number is not increased over the normal. In the interior of the graft neither the fibrocytes nor the undifferentiated mesenchymal cells are augmented. After eight days the transplant is surrounded by loose vascularized fibrous tissue. The regeneration within the graft itself is progressing and takes place in the reticulum and the lymphoid tissue of the peripheral zone as well as in the central zone. In some areas there is an outgrowth of endothelial cells of the sinuses toward the center of the piece, producing small spaces filled with blood and lymph cells. Many newly formed small and medium-sized lymphocytes are found, while the previously necrotic central zone is replaced by a great number of phagocytic polyblasts. Associated with the new formation of cells there is a new production of loose reticulum fibers. This process is still more evident after twelve days. After twenty-one days the transplanted tissue on the whole is apparently well preserved, displaying again the normal structure of spleen. The endothelial cells of the sinuses show no mobilization, but they still continue to grow out in some places. The undifferentiated mesenchymal cells and hemocytoblasts are not increased, but are present in their usual number. In the periphery of the transplant a conglomeration of eosinophilic leukocytes is found. The graft itself is surrounded by a large dense well vascularized layer of fibrocytes forming a connective tissue capsule.

Syngenesiotransplantation.—After two days the periphery as well as the center of the transplant has become necrotic to a considerable extent. Trabeculae are still evident, and sinuses and malpighian bodies are invaded for the most part by lymphocytes. In the center remnants of malpighian bodies are found with greatly injured lymphocytes. The living reticulum cells are transformed into free wandering cells, but no transformation into lymphocytes is observed. The small and medium-sized lymphocytes, so far as they are undamaged, have produced polyblasts of various shapes. The reticulum fibers have become swollen; they are spread out and partly dissolved. After four days the resorption processes in the graft have progressed still farther. The reticulum

· fibers are for the most part destroyed; the tissue itself is invaded by lymphocytes and a great many polyblasts coming from the blood stream of the host. The lymphoid cells of the transplant have become necrotic; the malpighian bodies damaged. Some of the reticulum cells are mobilized and transformed into phagocytic histiocytes invading the periphery. After eight days a considerable production of fibrocytes is seen surrounding the transplant, while histiocytes are found all over the graft. These occur chiefly at the periphery, and their number is diminished toward the central part. This finding, in addition to the fact that in earlier stages the histogenous polyblasts produced inside the transplant have become mostly necrotic, suggests that the majority of the histiocytes are of hematogenous origin from the host. Accordingly, after from twelve to twenty-one days, the graft is almost completely replaced by dense masses of connective tissue showing hard collagenous and softer reticulum fibers. Only very few remnants of trabeculae and sinuses with well preserved endothelium are noticeable. The proliferation of fibrocytes indicates that formative processes on the part of the antagonistic mesenchyme of the host lead to an overwhelming of the transplant by host elements.

Homeotransplantation.—As in the syngenesiotransplant, one notices here, also, as early as after two days numerous necrotic areas in the center of the homeotransplant. The reticulum fibers have obviously become dissolved, while the lymphocytes have been mostly destroyed. A certain resistance of the endothelial cells is found. The invasion of the graft by lymphocytes is pronounced. Masses of hematogenous polyblasts from the host are actively wandering toward the center, but no evidence of · any considerable reaction of the lymphoid, reticulum or endothelial cells of the graft can be detected. Necrotic remnants of malpighian bodies are still present, whereas in the periphery some trabeculae and sinuses are sometimes alive. Here and there a histogenous reaction on the part of the graft is noted. But as a rule all over the transplant it is the polyblasts coming from the vessels of the host which prevail, and they decrease in number toward the necrotic central zone. After eight days scanty remnants of the spleen are still evident in the periphery; the center has become completely necrotic. A great number of histiocytes are present showing phagocytosis. A layer of well vascularized connective tissue has surrounded the graft. After twelve days the transplant has been for the most part replaced by polyblastic histiocytes and fibrocytes with a formation of fibers growing toward the periphery. A striking ingrowth of capillaries is seen as well as a considerable migration of eosinophilic leukocytes into the graft. After twenty-one days these leukocytes are no longer visible. A perfect organization has taken place. However, in the periphery of the trans-

plant trabeculae and the last remnants of sinuses may still be recognized. A well vascularized connective tissue replaces the transplant.

COMMENT AND CONCLUSIONS

Autotransplants manifest a certain growth and full regenerative tendency, which is evident after twenty-one days. All the elements of the spleen are able to regenerate.

1. Endothelial cells show typical outgrowth in a lengthwise direction, forming new venous and lymph sinuses.

2. Reticulum cells have a marked power of resistance to injury connected with transplantation and produce phagocytes, but there is no evidence of their transformation into lymph cells.

3. Lymphocytes, so far as they remain alive, show two kinds of reaction:

(*a*) Some increase in number as a result of mitotic proliferation which leads to a new formation of small and medium-sized lymphocytes. This takes place especially in the malpighian bodies.

(*b*) Others undergo hyperplastic changes leading to the production of polyblasts. Later on these become fixed, new fibers being produced.

4. Hemocytoblasts and indifferent mesenchymal cells exert their power of further differentiation.

The graft itself is surrounded by a massive fibrous capsule, which, in general, is not usual in autotransplants.

In syngenesiotransplants and homeotransplants full regeneration was not noted. As to the origin of the phagocytes one has to consider as possible sources: (1) reticulum cells of the graft; (2) surviving lymphocytes of the graft; (3) nongranular white cells of the host; (4) preexisting histiocytes of the connective tissue of the host.

Without doubt, some of the histiocytes are formed from the transplanted reticulum cells. As to their derivation from preexisting lymphocytes, I [1a] have demonstrated that such a transformation certainly occurs. Many of these phagocytes in the earlier stages following transplantation were seen in the center proceeding toward the periphery, where they were destroyed. In later periods a new accumulation of these phagocytic cells was observed in the periphery, together with an invasion by leukocytes, at a time when the common lymphocytes had disappeared. Such observations indicate that a transformation of the invading lymphocytes and monocytes into histiocytes must have taken place. The number of preexisting monocytes in blood and lymph is too small to explain

1. Silberberg, M.: (*a*) Virchows Arch. f. path. Anat. **274**:820, 1930; (*b*) in Hirschfeld, Hans, and Hittmair, Anton: Handbuch der allgemeinen Hämatologie, Berlin, Urban & Schwarzenberg, 1932, vol. 1, p. 2.

completely the considerable number of polyblasts which are found in these grafts. Subsequently the graft is absorbed by these phagocytes, which at a still later time are transformed into fibrocytes. The pre-existing histiocytes of the host's connective tissue likewise are too few to explain the formative processes which take place within the graft.

According to Jaffe and Richter,[2] regeneration of lymphocytes in transplants of splenic tissue is effected mostly by hyperplastic reticulum cells. They did not, however, distinguish between the indifferent mesenchymal cells and the reticulum cells. Though a number of more recent experiments [3] have clearly shown that a transformation of lymphocytes and reticulum cells into polyblastic histiocytes is possible, a transformation of histiocytes into lymphocytes is not proved. While a development of lymph cells from the indifferent mesenchymal cells directly or by way of hemocytoblasts is quite possible, such an origin would be exceptional.

Marine and Manley [4] have pointed out that in autotransplants of spleen survival and growth are the rule. I noted a variable growth tendency. I used young guinea-pigs, which may account for the fact that I had better results with homeotransplants than the last named investigators. I could not find any evidence for their statement that removal of the spleen acts as a powerful stimulus to the growth of the graft. Considering the work done in tissue cultures one knows that the sharper the cut edges of a piece are the better is its growth in culture. It may be that grafts with many sharp cuts would show better growth than those with only a few cuts.

All the experiments made so far make it evident that tissue from hemopoietic organs has no marked resistance to transplantation (Loeb [5]), while there is no doubt that epithelial organs and their cells, as a rule, show better regenerative power. Ordinary connective tissue also has a high growth tendency. It may therefore be assumed that the tissue differential [5d] of epithelial and mature connective tissues is firm and constant, while that of the hemopoietic tissues, in spite of their natural variability, can be less readily maintained.

Whenever hemopoietic tissues are placed in certain mediums or even merely removed from their original position within the body one finds a definite result: In tissue cultures as well as in syngenesiotransplants

2. Jaffe, H. L., and Richter, M. N.: J. Exper. Med. 47:917, 1928.

3. Maximow, A., in von Möllendorff, Wilhelm: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 2, p. 1. Bloom, W., in Hirschfeld, Hans, and Hittmair, Anton: Handbuch der allgemeinen Hämatologie, Berlin, Urban & Schwarzenberg, 1932, vol. 1, p. 20. Silberberg.[1]

4. Marine, D., and Manley, O. T.: J. Exper. Med. 32:113, 1920.

5. Loeb, Leo: (a) Arch. Path. 12:203, 1931; (b) American Naturalist 65: 385, 1931; (c) Biol. Bull. 40:143, 1921; (d) Physiol. Rev. 10:547, 1930.

and homeotransplants of spleen all the various well characterized elements of this tissue undergo changes which finally lead to their transformation into fibrocytes, thus indicating a loss of their characteristic tissue differential.

Presumably the changes in the nature of the surrounding fluid medium which take place under these conditions, and perhaps also the lack of connection with the regulating influence of nerves, lead to the loss of the cellular characteristics of these mesenchymal cells. In forming fibrocytes the cells of the mesenchyme undergo the least specific differentiation. At the same time the reactions on the part of the host's mesenchyme prevail over the grafted hemopoietic tissues; the latter seem to elicit a much stronger homeotoxic reaction in the surrounding tissue of the host than do other transplanted tissues. In autotransplants, vascularization taking place and the body fluids remaining the same, the conditions for regeneration and growth are better; these grafts therefore remain alive a longer time, and their cells retain their specific character.

SUMMARY

Autotransplants of spleen manifest positive growth and full regenerative tendency.

The regeneration of this organ is characterized by a specific and remarkably balanced growth of all its different cells; it is usually completed within from sixteen to twenty-one days following transplantation.

In syngenesiotransplants and homeotransplants a full regeneration does not occur.

The cytologic observations reported here strongly suggest a transformation of lymphocytes into polyblasts and histiocytes.

Lymphoid and hemopoietic tissue, in general, have no marked resistance to transplantation.

It may be assumed that the tissue differential of epithelial organs and connective tissues is pronounced, while that of the hemopoietic tissues, in spite of the variability of their cells, is less marked. The results of transplantation and explantation of hemopoietic tissue may be explained by a loss of their tissue differential.

EFFECT OF HYPOPHYSECTOMY ON NATURAL RESISTANCE OF ADULT ALBINO RATS TO HISTAMINE POISONING

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The removal of the adrenal glands of rats is followed by a marked depression in the natural resistance to various toxins, poisons and bacterial and protozoan infections. The depression is associated to a greater degree with the loss of cortical than with that of medullary substance. This conclusion is based on the fact that the administration of epinephrine to adrenalectomized rats influences the resistance only to a slight degree,[1] whereas the administration of the adrenal cortical hormone may raise the resistance almost to the normal level.[2]

The work of Smith[3] and other investigators demonstrated that removal of the hypophysis in rats is followed by atrophy of the adrenal cortex as well as of the lymphatic tissue, gonads, thyroid gland and thymus. The atrophy of the adrenal cortex is, however, unassociated with changes in the medullary portion of the gland. The experiments reported in the present communication were undertaken to determine the effect of hypophysectomy on the natural resistance of rats to histamine poisoning and to correlate the anatomic changes in the adrenal gland with the changes in resistance. In the course of the work it was possible to determine that the posterior lobe of the hypophysis played no significant part in the maintenance of resistance to this poison.

While this work was in progress, Wyman and tum Suden[4] reported studies on the reactions of hypophysectomized rats to histamine. They observed that after total hypophysectomy the minimum lethal dose of histamine may or may not be reduced to one half.

1. Perla, D., and Marmorston-Gottesman, J.: Am. J. Physiol. **89**:152, 1929.

2. Perla, D., and Marmorston-Gottesman, J.: Proc. Soc. Exper. Biol. & Med. **28**:1022, 1931.

3. Smith, P. E.: Am. J. Anat. **45**:205, 1930.

4. Wyman, L. C., and tum Suden, Caroline: Am. J. Physiol. **109**:115, 1934.

METHOD OF INVESTIGATION

All the rats used in these studies were of an original Wistar Institute stock raised in our laboratory during a period of twelve years.[5] At the time of operation the rats used were between 3 and 4 months of age. The hypophysis was removed, according to the method described by Smith, through the ventral parapharyngeal route. The approach was through the nasopharynx. Precautions were taken in the postoperative care of the animals to maintain a high temperature, as hypophysectomized animals are very sensitive to cold.

Of seventy-six rats that were hypophysectomized completely or partially and that survived the operation, thirty-two on which there was no further intervention died in from two to eighty-eight days.[6] The remaining forty-four rats were given injections of varying amounts of histamine at varying intervals after hypophysectomy. Some of these animals were given repeated injections at long intervals of increasing amounts of histamine in an effort to determine the lethal dose. Autopsies were performed on all the rats, and the percentage weights of the liver, spleen, kidneys, thyroid gland, thymus, adrenals, ovaries, tubes and uterus, testes, seminal vesicles and prostate were determined. Sections of all the organs were studied histologically, and the hypophyseal region was sectioned and examined histologically for fragments. In many instances gross or microscopic fragments of the anterior lobe were noted, but in none were fragments of the posterior lobe observed. Under these circumstances the effect on the resistance of fragments of the anterior lobe could be estimated. The susceptibility of the rats to histamine poisoning was studied.

OBSERVATIONS

It was observed that loss of weight of the animal and atrophy of the testes were not reliable criteria of complete removal of the anterior lobe. In seven instances marked atrophy of the testes was observed in the presence of fragments of the anterior lobe of the hypophysis that were visible grossly. The adrenal glands showed striking changes within nine days after hypophysectomy. Hemorrhage appeared in the reticular zone of the cortex sometimes as early as the third day after hypophysectomy. The presence of cortical hemorrhage was noted in rats that showed no evidence of infection and received no histamine. In other rats large macrophages filled with hemosiderin[7] were observed between the cortex and the medulla. The hemorrhagic change in the adrenal cortex in adult rats after hypophysectomy[8] apparently precedes the atrophy of the cortex described by Smith.[8]

Transient diabetes insipidus, which disappeared within four or five days after the operation, was frequently noted in completely hypophysectomized rats. During this period the increased excretion of water was so marked that the fur of the rat was constantly wet about the genitalia. In partially hypophysectomized rats the increased excretion of water was not prominent, though an increase in the intake of water occasionally occurred. Accurate quantitative determinations were

5. The rats were of Bartonella-carrier stock. Smears of the blood and blood counts were made on a number of the animals in the course of the experiment, but the latent infection was apparently uninfluenced by hypophysectomy.

6. Extreme sensitivity to cold is a striking feature. A high mortality after hypophysectomy occurs if the temperature of the postoperative environment is permitted to drop below 80 F. Sensitivity to cold after this operation is even more striking than after adrenalectomy.

7. The pigment appeared blue with the Turnbull stain for iron pigment.

8. Perla, David: Proc. Soc. Exper. Biol. & Med. **32**:655, 1935.

not, however, consistently recorded. In a recent study Richter [9] reported transient diabetes insipidus in completely hypophysectomized rats and permanent diabetes insipidus in rats in which the posterior lobe and part of the anterior lobe were removed.

Effect of Hypophysectomy on Resistance of Rats to Histamine Poisoning One Week After Operation.—Within the first nine days thirty-three rats on which operation was performed died spontaneously. In eight instances local cellulitis at the site of operation occurred. In one purulent urethritis was present. Sixteen rats died of shock within forty-eight hours. In eight of the twenty-one animals surviving for from three to nine days there were hemorrhagic changes in the adrenal glands. Infection of the sinuses occurred in a few rats.

Twenty-three rats received histamine seven to nine days after operation in amounts varying from 150 to 500 mg. per kilogram of body weight. In nine of these animals fragments of the anterior lobe were observed at autopsy; in six the

TABLE 1.—*Effect of Hypophysectomy on Resistance of Rats to Histamine Poisoning Seven to Nine Days After Operation*

Operation	Number of Rats	Histamine, Mg. per Kg.	Number Survived	Number Killed
Complete hypophysectomy......	1	150	1	0
	3	200	1	2
	1	300	0	1
	1	400	0	1
Partial hypophysectomy.........	1	150	1	0
	6	200	6	0
	2	400	1	1*
	1	500	1	0
Exposure of hypophysis without removal	2	400	2	0
	1	600	1	0
	1	900	1	0
Normal controls..................	2	900	1	1
	2	1,000	0	2

* The adrenal glands were atrophic; the fragment of the anterior lobe of the hypophysis was minute.

hypophysis was completely removed, and in four the hypophysis was exposed but not removed (controls on which operation was performed). Four normal rats received 900 and 1,000 mg. of histamine per kilogram of body weight. As may be seen in table 1, the hypophysectomized rats were killed by histamine in amounts as small as 200 mg. per kilogram, whereas the controls survived injections of 900 mg. per kilogram. The resistance of the hypophysectomized rat one week after operation was about one-fifth that of the normal rat.

Effect of Hypophysectomy on Resistance to Histamine Poisoning from Two to Four Weeks After Operation.—In this group fifty-five tests were performed on thirty-eight rats, three of which were normal animals used as controls. In twelve instances the hypophysis was observed to be completely removed. In twenty-five instances fragments of the anterior lobe were noted, and in fifteen the hypophysis had been exposed but was not disturbed (controls on which operation was performed). The interval between the time of operation and that of injection varied from thirteen to twenty-eight days. The amount of histamine administered to the animals which had been operated on varied from 150 to 900 mg. per kilogram of body weight. The three normal animals used as controls received 900, 1,000 and 1,100 mg. of histamine per kilogram of body weight.

9. Richter, Curt: Am. J. Physiol. **110**:439, 1934.

As may be noted in table 2, the completely hypophysectomized rats were killed by histamine in doses as small as 300 mg. per kilogram of body weight, while the partially hypophysectomized rats survived doses of as much as 600 mg. per kilogram. The controls which were operated on and the normal controls survived the injection of as much as 900 mg. per kilogram of body weight. In one partially hypophysectomized rat that was killed by 600 mg. of histamine per kilogram, the adrenals were small, and evidence of old hemorrhage in the reticular zone of the cortex was seen. In another rat which was killed by 500 mg. per kilogram, the adrenals were also small, although a large fragment of the anterior lobe of the hypophysis was present. In one of two rats used as controls on which operation was performed and which died after receiving 900 mg. of histamine per kilogram, autopsy revealed a localized abscess in the neck at the site of operation.

TABLE 2.—*Effect of Hypophysectomy on Resistance to Histamine Poisoning Two to Four Weeks After Operation*

Operation	Number of Rats	Histamine, Mg. per Kg.	Number Survived	Number Killed
Complete hypophysectomy......	3	150	3	0
	2	200	2	0
	5	300	3	2
	1	400	0	1
	1	500	0	1
Partial hypophysectomy.........	3	150	3	0
	4	200	4	0
	7	300	7	0
	5	400	5	0
	3	500	2	1*
	2	600	1	1†
Exposure of hypophysis without removal	5	200	5	0
	1	300	1	0
	1	400	1	0
	2	500	2	0
	2	600	2	0
	2	800	2	0
	2	900	1	1
Normal controls..................	1	900	1	0
	1	1,000	0	1
	1	1,100	0	1

* The adrenal glands were small.
† Evidence of old hemorrhage in the cortex (reticular zone) and of recent hemorrhage was noted.

In two of the rats in which fragments of the anterior lobe of the hypophysis remained the adrenal cortex was atrophic, and in three the weight of the gland was only slightly less than normal. The testes were atrophic in three animals, but the seminal vesicles and prostate were definitely atrophic in six. The ovaries and tubes showed regressive changes in two animals. Apparently, then, atrophy of the gonadal tissue may occur in the presence of fragments of the anterior lobe of the hypophysis, and the cortex of the adrenal may also show involutional changes in the presence of such fragments.

From this experiment it appears that the minimum lethal dose of histamine for hypophysectomized rats from two to four weeks after operation is equivalent to about one-third the lethal dose for normal rats (table 2).

Effect of Hypophysectomy on Resistance to Histamine Poisoning from Five to Ten Weeks After Operation.—In this group forty-one tests were made on thirty-seven rats. Four of these tests were performed on normal rats used as controls,

eight on rats in which the hypophysis was completely removed, fifteen on rats in which fragments of the anterior lobe were noted at autopsy and fourteen on rats in which the hypophysis was exposed but not disturbed (controls on which operation was performed).

Two of the normal rats used as controls received 1,000 mg. and two 1,200 mg. of histamine per kilogram of body weight. The rats which had been operated on received histamine in doses ranging from 300 to 1,000 mg. per kilogram of body weight.

In table 3 it is shown that the completely hypophysectomized rats were killed by histamine in doses of 600 mg. per kilogram of body weight and above but that they survived smaller doses. The partially hypophysectomized rats survived doses

TABLE 3.—*Effect of Hypophysectomy on Resistance to Histamine Poisoning Five to Ten Weeks After Operation*

Operation	Number of Rats	Histamine, Mg. per Kg.	Number Survived	Number Killed
Complete hypophysectomy......	1	300	1	0
	2	400	2	0
	1	600	0	1
	2	800	0	2
	1	900	0	1
	1	1,000	0	1
Partial hypophysectomy.........	3	400	3	0
	4	600	3	1*
	4	700	0	4†
	2	800	0	2
	2	1,000	0	2
Exposure of hypophysis without removal	1	400	1	0
	1	600	1	0
	2	700	2	0
	2	800	2	0
	6	900	5	1
	2	1,000	1	1
Normal controls..................	2	1,000	1	1
	2	1,200	0	2

* The adrenal glands showed evidence of old and recent hemorrhages.
† Two of these were pregnant females—one with bronchopneumonia and one with atrophy of the adrenal glands.

as large as 600 mg. per kilogram of body weight. The rats used as controls on which operation was performed survived doses of as much as 1,000 mg. per kilogram of body weight, the operative procedure having no effect on the resistance to histamine. The normal animals used as controls survived injections of as much as 1,000 mg. per kilogram.

The adrenal glands of the completely hypophysectomized rats were found to be atrophic at autopsy, the cortex being smaller than normal.

Of the four partially hypophysectomized rats which were killed by 700 mg. of histamine per kilogram, two were pregnant at the time of injection; one had intercurrent bronchopneumonia and a localized abscess in the hypophyseal region; and the fourth, though possessing a fragment of the anterior lobe of the hypophysis, had atrophic adrenals. Of the partially hypophysectomized animals in which the adrenals were found to be of normal size, three survived a dose of 600 mg. per kilogram, and two were killed by injections of 800 mg. per kilogram. The fragments of the anterior lobe of the hypophysis noted at autopsy in this group of rats

were apparently viable and often showed evidence of hypertrophy. The presence of a fragment of the anterior lobe is sufficient to maintain a high degree of resistance to histamine unless the adrenals are atrophic (table 3).

In four of the group of rats on which tests were made from five to ten weeks after operation localized spontaneous infections were observed, all in the vicinity of the operative site. In two of the controls on which operation was performed localized abscesses were noted beneath the hypophyseal membrane, but not invading it. Neither animal died of histamine poisoning. In one of the partially hypophysectomized rats a small abscess was observed in the hypophyseal area, though not destroying the fragment.

It is evident that removal of the pituitary gland in rats from five to ten weeks prior to injection of histamine definitely diminishes the natural resistance of these animals to histamine to about one-half that of normal rats. Even in the presence of a small fragment of the anterior lobe there may be a marked decrease in adrenotropic substance in some instances, as indicated by atrophy of the adrenal cortex.

TABLE 4.—*Effect of Partial Hypophysectomy on Resistance to Histamine Poisoning Thirteen to Twenty-Six Weeks After Operation*

Operation	Number of Rats	Histamine per Kg. of Body Weight, Mg.	Number Survived	Number Killed
Partial hypophysectomy.........	2	600	1	1
	1	700	1	0
	3	800	2	1
	3	900	1	2
	1	1,000	0	1
Exposure of hypophysis without removal	1	900	0	1
	2	1,000	1	1
	2	1,100	0	2
Normal controls.................	1	900	1	0
	1	1,000	0	1
	1	1,100	0	1

Since the medulla is apparently unaffected by hypophysectomy, it is probable that the decrease in resistance to histamine after this procedure is associated with the cortical atrophy.

Effect of Partial Hypophysectomy on Resistance to Histamine Poisoning, from Thirteen to Twenty-Six Weeks After Operation.—In this group tests were made on eighteen rats. Of these three were normal. In ten rats fragments of the anterior lobe were noted, which in some instances were apparently hypertrophied to from one-third to two-thirds the size of the normal anterior lobe. The posterior lobe was absent in all of these animals. In five rats the hypophysis had been exposed but was undisturbed (controls on which operation was performed). In no instance was the hypophysis completely removed.[10] The partially hypophysectomized rats received from 600 to 1,000 mg. of histamine per kilogram of body weight. Five controls on which operation was performed received histamine in doses of from 900 to 1,100 mg. per kilogram. In table 4 it is shown that the partially hypophysectomized rats survived doses of as much as 900 mg. of histamine per kilogram, though in a few instances they were killed by smaller doses.

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10. Adult rats do not survive complete hypophysectomy as well as immature rats. At the time of this experiment we were not successful in keeping completely hypophysectomized rats alive for longer than three months.

It is striking that all the partially hypophysectomized rats surviving the injections of histamine possessed adrenal glands of normal size and appearance, even though in some instances the gonads or other tissues showed atrophic changes. The rats with atrophy of the adrenal glands were in all instances killed by histamine in amounts less than the minimum lethal dose for normal rats. However, in one instance a rat with apparently normal adrenals was killed by 800 mg. per kilogram.

Effect of Adrenal Cortical Hormone on the Natural Resistance of Hypophysectomized Rats to Histamine.—Since the decrease in resistance to histamine in hypophysectomized rats was associated with atrophic changes of the adrenal cortex, the effect of repeated injections of adrenal cortical hormone on the natural resistance of hypophysectomized rats to histamine poisoning was studied.

Seventeen totally hypophysectomized adult albino rats were divided into two groups. One group of nine hypophysectomized rats from five to eight days after operation were given intraperitoneal injections twice daily during a period of six days of adrenal cortical hormone [11] in amounts of 1 cc. daily for each rat (equivalent to 40 Gm. of fresh ox cortex). The other group of eight hyophysectomized rats received no cortical hormone. Six days after treatment was begun the rats treated with cortical hormone were given injections of histamine in amounts ranging from 600 to 800 mg. per kilogram of body weight. The untreated hypophysectomized rats received histamine in amounts ranging from 200 to 700 mg. per kilogram. Two rats used as controls on which operation was performed received 600 and 900 mg., respectively, of histamine per kilogram of body weight. Of four normal rats used as controls two received 900 mg. of histamine per kilogram and two 1,000 mg.

The rats treated with cortical hormone in most instances appeared to be improved clinically and gained in weight, though there was no anatomic effect on the atrophied adrenal cortex.

In table 5 it is shown that of the nine hypophysectomized rats treated with cortical hormone six survived injection of histamine in doses of from 600 to 800 mg. per kilogram of body weight and three were killed by doses of 700 and 800 mg. per kilogram.

The hypophysectomized rats not treated with cortical hormone and receiving injections of histamine were killed by doses as low as 200 mg. per kilogram of body weight and did not in any instance survive a dose of more than 400 mg. per kilogram.

The repeated injections of large amounts of adrenal cortical hormone raised the natural resistance of totally hypophysectomized adult rats to histamine poisoning in some instances almost to the level of that of normal rats (table 5). It will be noted that this increase in resistance was effected during the first two weeks after hypophysectomy, when the greatest decrease in natural resistance to histamine was observed to occur. Whereas the minimum lethal dose of histamine for hypophysectomized rats within this period varied from 200 to 300 mg. per kilogram of body weight, the hypophysectomized rats treated with cortical hormone during six days prior to the injection of histamine survived in many instances a dose of 800 mg. of histamine per kilogram of body weight. The effectiveness of the parenteral administration of any given amount of cortical hormone to hypophysectomized rats probably varies to some degree with the total amount of cortical hormone which may still be available in the involuted adrenal cortex.

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11. Extract of adrenal cortex (Parke, Davis & Co.).

TABLE 5.—*Effect of Repeated Injections of Adrenal Cortical Hormone on Natural Resistance of Hypophysectomized Adult Albino Rats to Histamine Poisoning* *

Rat	Sex	Operative Procedure	Interval Between Operation and Injection of Extract of Adrenal Cortex, Days	Interval Between Operation and Injection of Histamine, Days	Weight at Operation, Gm.	Weight at Beginning of Treatment with Cortical Extract, Gm.	Weight at Time of Injection of Histamine, Gm.	Amount of Histamine, Mg. per Kg. of Body Weight	Result	Comment
		Rats Treated with Extract of Adrenal Cortex								
1	M	Complete hypophysectomy	7	14	205	183	196	700	Survived	
2	F	Complete hypophysectomy	6	14	191	178	180	800	Survived	
3	M	Complete hypophysectomy	5	18	271	221	233	800	Died	
4	M	Complete hypophysectomy	5	13	144	140	183	700	Survived	
5	M	Complete hypophysectomy	8	14	165	140	145	700	Died	Survived 2 days after injection of histamine
6	M	Complete hypophysectomy	8	14	174	150	161	800	Survived	
7	F	Complete hypophysectomy	7	12	154	125	132	800	Survived	
8	M	Complete hypophysectomy	6	11	157	138	130	700	Died	Marked atrophy of adrenals
9	M	Complete hypophysectomy	5	11	149	140	122	600	Survived	
		Rats Not Treated with Extract of Adrenal Cortex								
11	F	Complete hypophysectomy	0	12	165	....	150	400	Died	
12	F	Complete hypophysectomy	0	13	152	....	117	400	Survived	
13	M	Complete hypophysectomy	0	54	205	....	170	700	Died	
14	F	Complete hypophysectomy	0	50	191	....	170	600	Died	
15	M	Complete hypophysectomy	0	11	179	....	160	200	Survived	
16	F	Complete hypophysectomy	0	12	152	....	124	300	Died	
17	F	Complete hypophysectomy	0	15	161	....	140	300	Died	
18	M	Complete hypophysectomy	0	16	180	....	138	400	Died	
19	M	Exposure of hypophysis without removal	0	14	210	....	192	900	Survived	
20	F	Exposure of hypophysis without removal	0	12	212	....	196	600	Survived	
21	F	Normal control	0	....	....	....	165	900	Survived	
22	M	Normal control	0	....	....	....	185	900	Died	
23	F	Normal control	0	....	....	....	160	1,000	Died	
24	M	Normal control	0	....	....	....	190	1,000	Died	

* All treated rats received 0.5 cc. of cortical hormone (equivalent to 20 Gm. of whole cortex) twice daily over a period of six days prior to the injection of histamine.

COMMENT

It has been demonstrated in previous communications and by the work of other investigators that removal of the adrenal glands is associated with marked depression in the natural resistance to toxins, poisons and bacterial and protozoan infections.[12] It was observed that resistance to histamine in adrenalectomized rats [13] may drop to one eighth that of the normal animal, and it was suggested that histamine may be used as a gage of adrenal insufficiency.

In subsequent work by Perla and Marmorston it was found that the level of resistance to histamine after adrenalectomy could be raised almost to that of the normal animal by the administration of extract of the adrenal cortex [2] containing the life-prolonging hormone of the adrenal cortex. This suggested that the depression in the resistance to histamine after adrenalectomy was essentially due to loss of cortical function, particularly since administration of the medullary secretion, epinephrine, had only a slight effect in raising the resistance to histamine poisoning in adrenalectomized rats.[1]

The extensive work of Smith [14] and other investigators [15] demonstrated that hypophysectomy in rats is followed by atrophy of the adrenal gland.[16] This suggested to us that a decrease in the resistance to histamine might follow hypophysectomy in rats.

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12. Perla, David, and Marmorston, J.: Arch. Path. **16**:379, 1933.

13. Marmorston-Gottesman, J., and Gottesman, J.: J. Exper. Med. **47**:503, 1928.

14. Smith, P. E.: Anat. Rec. **32**:221, 1926; J. A. M. A. **88**:158, 1927; footnote 3.

15. (*a*) Richter, C. P., and Wislocki, G. B.: Am. J. Physiol. **95**:481, 1930. (*b*) Evans, H.; Meyer, K.; Simpson, M. E., et al.: The Growth and Gonad-Stimulating Hormones of the Anterior Hypophysis, vol. XI, Memoirs of the University of California, Berkeley, Calif., University of California, 1933. (*c*) Collip, J. B.; Selye, H., and Thomson, D.: Nature, London **131**:56, 1933.

16. Similar observations have been described in the tadpole (Pigmentary Growth and Endocrine Disturbances Induced in the American Tadpole by the Early Ablation of the Pars Buccalis of the Hypophysis, American Anatomical Memoirs, no. 11, Philadelphia, Wistar Institute of Anatomy and Biology, 1920, p. 151. Atwell, W. F.: Proc. Soc. Exper. Biol. & Med. **29**:621, 1932) ; in the rabbit (Ikeda and Kusonoki, J.: Jap. J. Obst. & Gynec. **15**:213, 1932; Folia endocrinol. japon. **3**:34, 1927), and in the dog (Houssay, B. A., and Sammartino, R.: Compt. rend. Soc. de biol. **114**:717, 1933). In man under conditions associated with hypofunction of the hypophysis and in Simmond's disease, the adrenal glands are atrophic (Falta, W.: Erkrankungen der Blutdrüsen, Berlin, Julius Springer, 1913; Cushing, H., and Davidoff, L. M.: The Pathological Findings in Four Autopsied Cases of Acromegaly with a Discussion of Their Significance, Monogr. 22, Rockefeller Institute for Medical Research, 1927, p. 1; Erdheim, J.: Beitr. z. path. Anat. u. z. allg. Path. **62**:302, 1916, and Simmonds, M.: Deutsche med. Wchnschr. **45**:482, 1919). The atrophy of the adrenal cortex in hypophysectomized rats may be repaired by daily homotransplants of hypophysis (Smith [3]), by the injection of

Hence the present studies were undertaken, and it was found, as anticipated, that a depression in the natural resistance of adult albino rats to histamine poisoning occurred after hypophysectomy.[17] The resistance was reduced to a point as low as one fifth that of normal rats, the period of greatest susceptibility to histamine poisoning occurring from about one to two weeks after operation. In the later weeks the minimum lethal dose of histamine for hypophysectomized rats was increased to from about one third to one half that for the normal rat. The decrease in resistance was associated with hemorrhage into the reticular zone or with atrophy of the cortex of the adrenals. Partial hypophysectomy, in which the posterior lobe was destroyed but fragments of the anterior lobe remained, was also associated with a decrease in the natural resistance to histamine when involutional changes of the adrenal cortex occurred.

That the decrease in resistance to histamine after hypophysectomy is associated with the removal of the anterior lobe and not with that of the posterior lobe is indicated by the fact that the rats in which the posterior lobe was completely removed but in which a large fragment of anterior lobe remained survived injections of histamine in nearly the same amounts as did normal animals, provided involutional changes were not present in the adrenal cortex.

Since hypophysectomy results in atrophy of the adrenal cortex without producing noticeable changes in the adrenal medulla, the depression in natural resistance to histamine after this operation is probably not attributable to disturbance of medullary function but is probably associated with cortical atrophy induced by the withdrawal of the adrenotropic hormone of the anterior lobe of the hypophysis.

This hypothesis is corroborated by the finding that administration of adrenal cortical hormone raised the natural resistance of hypophysectomized rats (just as it does of adrenalectomized rats [2]) to histamine poisoning, in some instances almost to the normal level.

SUMMARY

The natural resistance to histamine was depressed in completely hypophysectomized rats from one to ten weeks after operation. The minimum lethal dose was from one fifth to one third that for normal rats.

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potent extracts of the growth-regulating hormone (Evans, Myers and Simpson [15b]) or of the adrenotropic hormone (Anderson, E. M.; Thomson, D. L., and Collip, J. B.: Lancet **2**:347, 1933). The administration of extract of the adrenal cortex in hypophysectomized rats does not prevent or repair the atrophy of the cortex (Shumacker, H. B., and Firor, W. M.: Endocrinology **18**:676, 1934).

17. It is interesting that, although hypophysectomized rats demonstrated a marked decrease in resistance to histamine, this depression was about one half as severe as that occurring after adrenalectomy in rats.

This decrease in resistance was associated with hemorrhage into, or atrophy of, the inner zone of the cortex of the adrenal.

Rats in which the posterior lobe and most of the anterior lobe were removed showed a similar decrease in resistance. In these instances atrophic changes in the adrenal cortex occurred. When a large fragment of anterior lobe remained there was no depression of resistance to histamine and the adrenal glands were normal.

Repeated injections of large amounts of adrenal cortical hormone raised the natural resistance of totally hypophysectomized adult rats to histamine poisoning. In some instances the resistance was raised almost to the level of that of normal rats.

The decrease in natural resistance to histamine after hypophysectomy in the rat is probably secondary to the atrophic changes in the adrenal cortex induced by the withdrawal of the adrenotropic hormone of the anterior lobe.

PROTEOLYTIC ENZYMES OF MONOCYTIC AND POLYMORPHONUCLEAR PLEURAL EXUDATES

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AND

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With the development of the conception that the reticulo-endothelial system is the site of antibody production,[1] the attention of immunologists has been directed toward a more intensive study of the cellular defense mechanisms of the body. A reinvestigation of the phenomenon of inflammation from this point of view led Opie[2] and Menkin[3] to conclude that this process is largely one of defense. Gay, Clark and Linton[4] showed that there are definite differences in the defensive properties of the various types of cells in an exudate. Thus, only the monocytes (clasmatocytes) are concerned in protection against micro-organisms like the streptococcus and the pneumococcus. The polymorphonuclear cells, on the other hand, not only may be ineffective, but may actually serve as a culture medium for these bacteria. Observations were also made by Opie, Barker and Dochez[5] and later by Jobling and Petersen[6] on the enzymes of the cells of inflammatory exudates and on the rôle of the so-called enzyme-antienzyme balance in infection and immunity.

The development in recent years of more accurate physicochemical methods and of new points of view in the study of enzymes, particularly through the work of Northrop,[7] Willstätter[8] and Waldschmidt-Leitz[9]

From the Research Laboratories of the Mount Zion Hospital.

1. Jaffé, R. H.: Physiol. Rev. 11:277, 1931.

2. Opie, E. L.: J. Immunol. 17:329, 1929.

3. Menkin, V.: Arch. Path. 12:802, 1931; Arch. Int. Med. 48:249, 1931.

4. (a) Gay, F. P., and Clark, A. R.: Arch. Path. 1:847, 1926. (b) Gay, F. P.; Clark, A. R., and Linton, R. W.: ibid. 1:857, 1926. (c) Linton, R. W.: ibid. 6:615, 1928.

5. Opie, E. L.: Physiol. Rev. 2:552, 1922; J. Exper. Med. 7:316, 1905; 8:410 and 536, 1906; 9:391, 414 and 515, 1907; 10:419, 1908; New York Path. Soc. 7-8:187, 1907; Bull. Johns Hopkins Hosp. 19:115, 1908. Opie, E. L., and Barker, B. I.: J. Exper. Med. 9:207, 1907; 10:645, 1908; 11:686, 1909. Opie, E. L.; Barker, B. I., and Dochez, A. R.: ibid. 13:162, 1911. Barker, B. I.: ibid. 10:666, 1908. Dochez, A. R.: ibid. 11:718, 1909.

6. Jobling, J. W., and Petersen, W. F.: Bull. Johns Hopkins Hosp. 26:356, 1915.

7. Northrop, J. H.: J. Gen. Physiol. 16:41, 1932. Kunitz, M., and Northrop, J. H.: J. Gen. Physiol. 17:591, 1934.

8. (a) Willstätter, R.; Bamann, E., and Rohdewald, M.: Ztschr. f. physiol. Chem. 188:107, 1930. (b) Willstätter, R., and Rohdewald, M.: ibid. 204:181, 1932.

9. Waldschmidt-Leitz, E.: Physiol. Rev. 11:358, 1931.

and their associates, suggested a reinvestigation of this subject. The present communication is concerned with the proteolytic enzymes of exudates of the monocytic and polymorphonuclear types which had been produced in the pleural cavities of normal rabbits. It is the first of a series of papers on the relation between the enzymic hydrolysis and the bacteriolysis accomplished by these exudates and on the possible effects of various bacterial toxins, carbohydrates and other substances as activators and inhibitors of these processes.

REVIEW OF THE LITERATURE

The investigations of Opie, Barker and Dochez revealed that polymorphonuclear cells obtained from pleural inflammatory exudates of dogs contain an enzyme, leukoprotease, which digests proteins in both neutral and alkaline reactions. Cells of the mononuclear series, on the other hand, have a lymphoprotease which acts only in weakly acid reaction. Husfeldt [10] reported two proteinases, cathepsin and trypsin, in neutrophils which had been obtained from a man with myelogenous leukemia. The first of these represents the enzyme which is active during autolysis; it digests proteins from p_H 3 to 7; the optimal digestion of casein and edestin is at p_H 4.3 and 5.2, respectively. The second enzyme, leukocytic trypsin, begins to function at p_H 4 and increases in activity with growing alkalinity of the solution. A third enzyme, dipeptidase, which is present in mixed leukocytes of normal human blood, digests the dipeptides, alanyl-glycine and leucyl-glycine, with optimal activity at p_H 7.2 and 8.1, respectively, and the tripeptide, alanyl-glycyl-glycine, at p_H 7.3. These peptidases were further subdivided by Wald-schmidt-Leitz [9] into a carboxy-polypeptidase and an amino-polypeptidase.

Kleinmann and Scharr [11] reported that hydrogen sulphide and hydrocyanic acid increase the proteolytic activity of the cathepsin of rabbit leukocytes, but only if gelatin is used as a substrate; no activation is observed with casein or edestin. The enzyme content of leukocytes varies somewhat with the species of animal used. Thus Willstätter, Bamann and Rohdewald [8a] found the leukocytes of the dog to be richer in trypsin than those of the horse. Moreover, in the former, trypsin is fully active, while in the latter activation is necessary.

The proteolytic enzymes of serum were likewise investigated several years ago by Opie and his associates. More recently Stephan and Wohl [12] and Kleinmann and Scharr [11b] have increased knowledge of this subject. Two proteinases were observed in the serums of rabbits and horses: a cathepsin associated with the globulin fraction and a tryptic enzyme bound to the albumin of the serum and liberated only when adsorbed on another protein such as casein. Enterokinase has no effect on either the bound or the free trypsin of serum. Heating to 56 C. or adjusting the reaction to p_H 5 or less [13] causes serums to lose their antitryptic power.

METHODS OF INVESTIGATION

Monocytic exudates were produced in the pleural cavities of rabbits by the injection of light mineral oil or a liquid petrolatum, specific gravity from 0.88 to 0.89,

10. Husfeldt, E.: Ztschr. f. physiol. Chem. **194**:137, 1931.

11. (*a*) Kleinmann, H., and Scharr, G.: Biochem. Ztschr. **251**:275, 1932; (*b*) **252**:145, 1932.

12. Stephan, R., and Wohl, E.: Ztschr. f. d. ges. exper. Med. **24**:391, 1921.

13. Weiss, C.: J. Infect. Dis. **41**:467, 1927.

at 25 C.[14] After an interval of five days, the animals were exsanguinated by bleeding from the carotid arteries under ether anesthesia, and the exudates were removed by opening the thoracic cavity. Five rabbits usually yielded about 75 cc. of exudate including about 45 cc. of oil, which was discarded. The exudate was divided into two equal parts, one of which was immediately separated by centrifugation into cells and supernatant fluid, while the other was placed in the refrigerator and tested as "total exudate" on the following day. The sedimented cells were washed four times with physiologic solution of sodium chloride in order to remove traces of serum proteins, then extracted over night in distilled water at refrigerator temperature (10 C.) and again centrifugated to remove the cellular residue or stroma. The latter was examined separately. The aqueous cellular extracts and the supernatant fluids were diluted with sufficient distilled water (about 96 cc.) to provide 2 cc. of enzyme material for each test tube.

Differential counts of the exudates made with the aid of Wright's stain and a supravital technic (neutral red used) showed about 90 per cent mononuclear cells, many of which were clasmatocytes. A few neutrophils were seen inside the cytoplasm of the monocytes, and a number of the cells contained oil in their cytoplasm. Several mesothelial cells and fibroblasts were also recognized. For comparison, polymorphonuclear exudates were produced by injecting a mixture of 5 per cent aleuronat and 3 per cent starch and removed after twenty-four hours. Differential counts of this exudate showed from 90 to 93 per cent polymorphonuclear cells and from 7 to 10 per cent clasmatocytes or histiocytes.

As substrate, 2 cc. of 2 per cent iso-electric gelatin was employed. This quantity was adequate, since on complete hydrolysis its total combining capacity in the titration in the presence of formaldehyde is 36 cc. of hundredth-normal sodium hydroxide, according to Cohn.[15] In some experiments a 2 per cent solution of purified casein was also used.

In order to determine the optimal p_H for autolysis or digestion, aliquot portions of each fraction of the exudate were placed into pyrex test tubes 25 by 150 mm. in size and adjusted by means of either hydrochloric acid or sodium hydroxide to p_H 2, 3 and so on up to p_H 10 at intervals of 1 p_H unit with the aid of the glass electrode of de Eds.[16] No buffers were employed, since it was not desired to introduce substances which might interfere with enzyme action. The total volumes were adjusted with distilled water to 10 cc. and the tubes placed in an air incubator at 37 C. for twenty-four hours. Determinations of p_H made at this time revealed no serious changes; the alterations seldom exceeded 1 p_H unit. Filtrations were done by the method of Northrop[17]: After adding neutral formaldehyde, the material was adjusted to p_H 6, and the titration carried to p_H 9 by means of hundredth-normal sodium hydroxide. Standard color tubes were used as guides in determining the end-points. The titration values of material which had been previously boiled for thirty minutes in order to destroy the proteolytic enzymes were subtracted from the corresponding values for material which was boiled just before titration. In determining the digestion of gelatin by an enzyme, the values obtained for autolysis were subtracted from the corresponding uncorrected figures obtained by titration in the presence of formaldehyde.

14. Lucké, B.; Strumia, M.; Mudd, S.; McCutcheon, M., and Mudd, E. B. H.: J. Immunol. **24**:455, 1933.

15. Cohn, E. J.: Ergebn. d. Physiol. **33**:781, 1931; tables on pp. 870 and 871.

16. de Eds, F.: Science **78**:556, 1933.

17. Northrop, J. H.: J. Gen. Physiol. **9**:767, 1926.

THE AUTOLYSIS OF PLEURAL EXUDATES

As seen in chart 1 A, an aqueous extract of rabbit monocytes of inflammatory origin contains a peptic enzyme which permits autolysis in acid reaction between (approximately) p_H 2 and 5, the optimum being at p_H 3. There is no evidence of cathepsin or of trypsin. An aqueous extract of polymorphonuclear cells (chart 2 A), on the other hand, shows activity in the region of p_H 3, 5.4 and 8, suggesting peptic, catheptic and tryptic types of digestion (autolysis). The supernatant fluid of a monocytic exudate freed immediately from its cellular elements (chart 1 B) shows tryptic digestion from about p_H 7 to 10, with an optimum at about p_H 8 and a slight amount of peptic action at p_H 3. The serous portion of a polymorphonuclear exudate (chart 2 B) behaves similarly but shows a little more peptic activity.

When an aqueous extract of monocytic cells is allowed to autolyze in the presence of the corresponding supernatant fluid, the former exerts strong inhibitory action on the latter from about p_H 10 to 7 (chart 1 C). A slight stimulation of cathepsin occurs between p_H 4.5 and 6.5. In the peptic range, between p_H 2 and 4, there is definite inhibitory action exerted by the supernatant fluid on the extract of the monocytes.[18] In the case of the polymorphonuclear cells, antitryptic action by the fluid is similarly evident. There is, however, inhibition of catheptic activity from p_H 5 to 7 with augmentation of peptic hydrolysis from p_H 2 to 4 (chart 2 C).

Since Gay and his co-workers discovered that the fluid separated from a pleural exudate after a delay of several hours loses its bactericidal power, it was of interest to ascertain how the proteolytic enzymes behave under similar circumstances. It was observed that when centrifugation of a polymorphonuclear exudate is delayed for twenty-four hours, the resulting fluid shows no cathepsin. Under similar circumstances the supernatant fluid of a monocytic exudate (table 1) acquires additional peptic activity, owing probably to autolysis of some of the cells.

When either a monocytic or a polymorphonuclear exudate was tested "whole," that is, without separation of leukocytes from the fluid, the autolysis accomplished in the regions of pepsin and cathepsin was greatly increased. The monocytic exudate showed, however, definite evidence of antitryptic action, since the level of the curve fell below that of the supernatant fluid or of the algebraic sum of the two constituents. The whole polymorphonuclear exudate did not show this phenomenon.
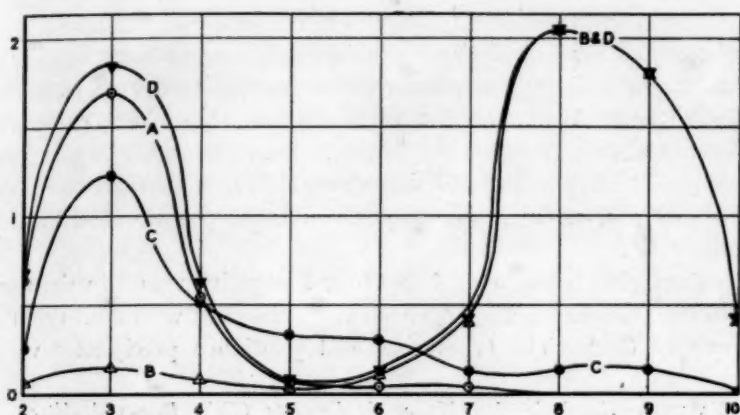
18. Hamburger, W. W.: J. Exper. Med. **14**:535, 1911.

Chart 1.—Autolysis of an inflammatory monocytic exudate at various p_H values: *A*, an aqueous extract of the cells; *B*, the supernatant fluid; *C*, both of these undergoing autolysis together (after being reunited); *D*, the algebraic sum of *A* and *B*, each undergoing autolysis separately. The numbers at the left represent values obtained by titration in the presence of formaldehyde, expressed in cubic centimeters of hundredth-normal sodium hydroxide. The numbers at the bottom represent the initial p_H values.
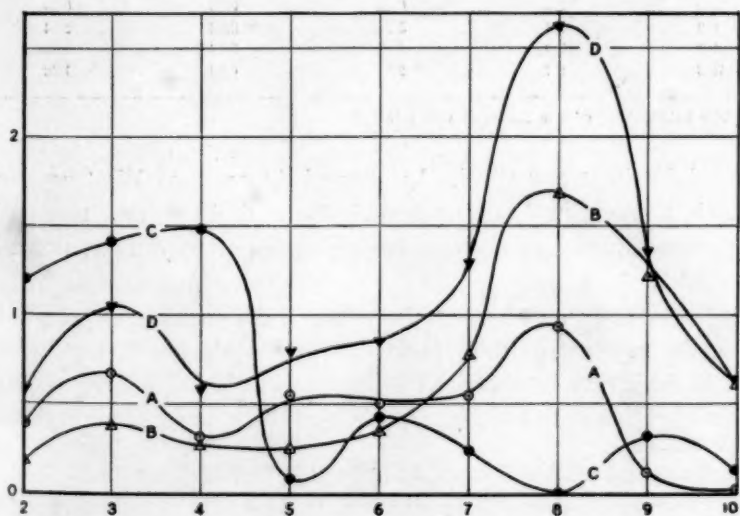
Chart 2.—Autolysis of an inflammatory polymorphonuclear exudate at various p_H values. See legend of chart 1 for explanation.

THE DIGESTION OF GELATIN BY PLEURAL EXUDATES, WITH
OBSERVATIONS ON RESYNTHESIS

The curves for hydrolysis of gelatin by aqueous extracts of cells of the monocytic and polymorphonuclear series are similar to those for autolysis (chart 3 *A*). Pepsin, cathepsin and trypsin are present in the latter and only pepsin in the former. Both supernatant fluids show tryptic activity, but in the acid range they differ, in that the monocytic type shows absence of peptic enzyme while the polymorphonuclear is slightly active.

In the region between p_H 4 and 6 or 7 negative values in the titration in the presence of formaldehyde were observed with both types of supernatant fluid. The tubes contained gelatinous precipitates which

TABLE 1.—*Autolysis of a Monocytic Exudate (Cells Removed After Twenty-Four Hours)*

| | | Values Obtained by Titration in the Presence of Formaldehyde (Cc. of 100th-Normal NaOH) | | |
| | | Total | Boiled | Enzyme |
Initial p_H*	Final p_H	Exudate	Control	Activity
2.0	2.1	1.14	0.80	0.34
3.0	3.4	1.86	0.76	1.10
4.0	4.1	1.03	0.59	0.44
5.0	5.1	0.53	0.48	0.05
6.0	6.0	0.45	0.42	0.03
7.0	7.0	0.68	0.45	0.23
8.0	7.3	3.27	0.53	2.74
9.0	7.9	3.43	0.79	2.64
10.0	8.7	2.31	0.89	1.42

* The original p_H of the material was 6.12.

were soluble in strong alkali. In view of the work of Wasteneys and Borsook,[19] Voegtlin[20] and others,[21] these findings are tentatively accepted as evidence of reversed enzyme action or resynthesis (plastein formation).

It is also to be noted (chart 3 *C*) that in the presence of gelatin the monocytic supernatant fluid exerts no antitryptic action on the cell extract. This may be explained by the observation of Northrop[22] that gelatin combines with the antitryptic or inhibitor substance. However,

19. Wasteneys, H., and Borsook, H.: Physiol. Rev. **10**:110, 1930.

20. Voegtlin, C.; Maver, M. E., and Johnson, J. M.: J. Pharmacol. & Exper. Therap. **48**:241, 1933.

21. Blagowestschenski, A. W., and Jeremejew, G. W.: Biochem. Ztschr. **270**:66, 1934.

22. Northrop, J. H.: J. Gen. Physiol. **4**:261, 1922.

since the antitryptic action persists when gelatin is exposed to the combined action of polymorphonuclear cells and fluid, an alternate hypothesis is suggested; namely, that resynthesis and hydrolysis proceed simultaneously, the curves representing the algebraic sum of these two separate effects. It is also possible that the fluid of a polymorphonuclear exudate contains much more inhibitor substance than the aqueous extract of monocytes. Hence, the quantity of gelatin employed in the test may not have been sufficient to combine with all of the inhibitor substance present. This subject, however, requires further investigation.

When centrifugation of either a monocytic or a polymorphonuclear exudate is delayed for twenty-four hours, the resulting fluid shows absence of catheptic activity and of resynthesis. Tryptic digestion seems to be markedly increased, suggesting an inactivation of the inhibitor of trypsin or a release or activation of the enzyme. When centrifugation of a monocytic or polymorphonuclear exudate is entirely omitted
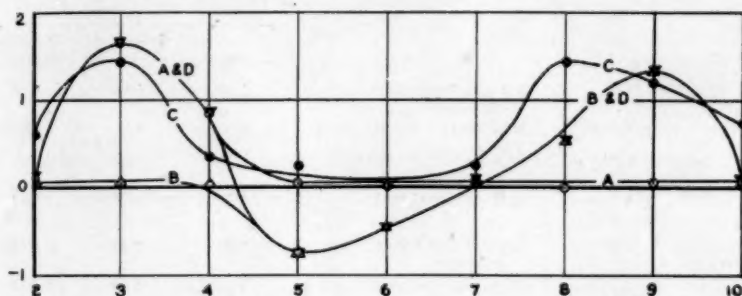
Chart 3.—Digestion of gelatin by a monocytic exudate at various p_H values. See legend in chart 1 for explanation.

and the whole exudate is tested, cathepsin is present, but resynthesis is not evident. In the region between p_H 2 and 4 there is evidence of diminished peptic activity in the case of the monocytic exudate and of strong activation in the case of the polymorphonuclear type.

The amount of gelatin hydrolyzed by extracts of either polymorphonuclear or monocytic cells depends on the amount of enzyme supplied. Similarly the amount of resynthesis varies directly with the amount of fluid present. The quantity of gelatin available as substrate did not affect the extent of resynthesis; double the amount usually employed gave similar results. Hence the protein resynthesized is probably derived from the protein-split products of the inflammatory exudate. Since gelatin, but not casein, combines with the so-called inhibitory substance [22] and helps to activate serum cathepsin,[11b] it was interesting to find absence of resynthesis in the presence of casein.

A dipeptide, leucyl-glycine, was also exposed to the action of various fractions of a monocytic exudate at p_H 3, 5.5 and 8.5. Both the fluid

and the whole exudate hydrolyzed this dipeptide at p_H 8.5. At p_H 5.5
negative values were obtained by titration in the presence of formalde-
hyde with the former, suggesting that resynthesis can occur in the
presence of a dipeptide as well as in that of gelatin.

THE PRESENCE OF MUTUALLY ANTAGONISTIC LYO-ENZYME AND DESMO-ENZYME IN MONOCYTES AND POLYMORPHONUCLEAR CELLS

Willstätter, Bamann and Rohdewald [8a] demonstrated that the stroma
remaining after extraction of leukocytes with glycerin is actively proteo-
lytic, and designated this bound enzyme as "desmo-enzyme" while the
extractable portion was termed "lyo-enzyme." In the present communi-

TABLE 2.—*Mutual Inhibition of Leukocytic Lyo-Enzyme and Desmo-Enzyme*

| Type of Cell | p_H | Preparation | Values Obtained by Titration in the Presence of Formaldehyde (Cc. of 100th-Normal NaOH) | | |
			Autolysis	Digestion of Gelatin	Digestion of Casein
Monocytes	3.0	Stroma and cell extract combined	1.10	5.00	0.60
	3.0	Mathematical sum	4.75	4.10	3.20
	8.5	Stroma and cell extract combined	0.20	0.00	0.00
	8.5	Mathematical sum	0.15	0.05	0.10
Polymorpho-nuclear cells	3.0	Stroma and cell extract combined	0.20	3.20	0.40
	3.0	Mathematical sum	1.70	3.50	4.50
	8.5	Stroma and cell extract combined	0.50	3.40	0.80
	8.5	Mathematical sum	2.65	2.90	3.35

cation we confirm and extend these observations. Our findings
indicate that aqueous extracts of rabbit monocytes and polymorpho-
nuclear cells contain more desmopepsin than lyopepsin. This was
observed during autolysis as well as during digestion of casein or
gelatin at p_H 3. The polymorphonuclear cells possess a comparatively
large amount of desmo-enzyme of the tryptic type in their stroma.
Since we have shown that rabbit monocytes contain no lyotrypsin, it
was interesting to find absence also of desmotrypsin in these cells.

Willstätter's findings of a mutual antagonism between leukocytic
lyotrypsin and desmotrypsin were also confirmed. The phenomenon was
observed with monocytes at p_H 3 and with polymorphonuclear cells at
both p_H 3 and 8.5. The substrates employed were casein or the proteins
associated with enzyme itself (autolysis). With gelatin the antagonism
was not apparent, possibly because this protein combines with the inhibit-
ing substance, as suggested by Northrop [22] (table 2).

COMMENT

It is a well known fact that during the development of an inflammatory reaction polymorphonuclear and monocytic phagocytes respond to the effects of the irritant. While the exact mechanism responsible for this phenomenon is not known, the work of Abramson [23] suggests that the migration of leukocytes to a point of injury may in part be dependent on the existence of a difference in potential between the injured and the relatively normal tissues. There is, moreover, an increase in the permeability of the blood vessels which permits the passage, in addition to the white blood cells, of an exudate containing serum proteins and fibrin. The digestion and absorption of these elements is accomplished by means of the proteolytic enzymes of the exudate. Owing to the concomitant apppearance of substances which inhibit proteolysis, it is necessary to study their behavior and also the rôle of various activators and accelerators of enzyme action during the stages of an infection. Although several investigators, including Opie [5] and Jobling, Petersen and Strouse,[6] have made important contributions in this field, much still remains to be learned. For example, what is the relation between the conditions favoring enzymic digestion and the capacity of the exudate to destroy bacteria? Do bacterial toxins, autolysates, specific carbohydrates, etc., interfere with or stimulate the resolution of an exudate? Is it possible to regulate this mechanism at will during an infection? Our investigations were begun with the hope of answering some of these questions.

In the present publication we confirm and extend Opie's observations concerning important differences in the behavior of monocytes (clasmatocytes) and polymorphonuclear cells derived from pleural inflammatory exudates. Whereas the monocytes contain only one proteinase, pepsin, the polymorphonuclear cells have pepsin, cathepsin and trypsin. The supernatant serous fluids derived from these exudates also differ in that the former type contains a substance inhibiting peptic digestion while the latter contains one enhancing the peptic activity of the leukocytes. Moreover, in the case of the polymorphonuclear exudate, it is the supernatant fluid which inhibits the tryptic activity of the cells, while in that of a monocytic exudate it is the cells which inhibit the tryptic autolysis of the supernatant fluid.

In addition to this, there is an inhibitory mechanism which concerns the leukocytes themselves. This was first observed by Willstätter, Bamann and Rohdewald,[8a] who showed the presence of a mutually antagonistic extractable enzyme, or lyo-enzyme, and bound enzyme, or desmo-enzyme, in the leukocytes. This is confirmed, and their presence in both monocytes and polymorphonuclear cells of inflammatory exudates demonstrated.

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23. Abramson, H. A.: J. Exper. Med. **46**:987, 1927.

Reference has been made to important differences which Gay and Clark [4a] observed in the bactericidal powers of these two types of exudates. Briefly, the monocytes can destroy streptococci and pneumococci, whereas the polymorphonuclear cells cannot. Exactly how this phenomenon is related to the differences in their enzyme action remains to be determined.

While the exact immunologic significance of the antienzyme substances in the blood or serous fluids has not been definitely established, the work of Wright [24] suggests that the antitryptic substance is related to the development of resistance to infection. It is necessary to confirm this and to determine whether the phenomenon has general application. Our observations to the effect that a cellular extract and its corresponding fluid at times inhibit each other's proteolytic activities while at other times they augment each other are strikingly similar to those of Mackie, Finkelstein and van Rooyen,[25] who showed that the bactericidal power of a serum-leukocyte mixture may under certain conditions be greater than the sum of the separate activities of the serum per se and the leukocytes per se while under other conditions normal whole blood and serum-leukocyte mixtures are inferior to serum in bactericidal power.

During the course of this study it was also observed that while gelatin was being digested by the fluid of either a polymorphonuclear or a monocytic exudate there was a decrease in carboxylic groups as evidenced by negative values obtained by titration in the presence of a formaldehyde between p_H 4.5 and 5.5 (or 6). A similar phenomenon was observed during the digestion of a dipeptide, leucyl-glycine, by the fluid of the monocytic type of exudate. In view of the work of several authors,[26] this is suggestive of a resynthesis of the protein-split products present in the exudate due to a reversal of catheptic action. Whether a similar process may, under proper conditions, go on in vivo during an inflammatory process is under investigation in this laboratory. Should this phenomenon occur, it might help to explain some phases of the problem of delayed resolution in pneumonia as well as the production of adhesions and of organization.

A delay in separation of an exudate into its constituents (cells and fluid) causes an inactivation of the trypsin inhibitor or a release or an activation of trypsin. It also makes conditions unfavorable for resynthesis. Gay and Clark [4a] showed that a supernatant fluid loses its bac-

24. Wright, A. E.: Brit. M. J. **2**:629, 1915.

25. Mackie, T. J.; Finkelstein, M. H., and van Rooyen, C. E.: J. Hyg. **32**: 494, 1932; J. Path. & Bact. **39**:89, 1934.

26. Wasteneys and Borsook.[19] Blagowestschenski and Jeremejew.[21] Voegtlin, Mayer and Johnson.[20]

tericidal power if it is permitted to stay in contact with its cells for
several hours before being tested. The question naturally arises: Do
conditions which decrease bactericidal action also favor resynthesis in
an inflammatory exudate, and hence delayed resolution?

It was pointed out in a foregoing paragraph that the optimal p_H for
peptic digestion by monocytes or polymorphonuclear cells is 3. This is
the p_H which Rous [27] found inside of these cells. The cells can main-
tain this intracellular acidity in spite of the alkalinity of the surrounding
fluid by virtue of the presence of dissolved carbon dioxide.[28] Since
bacteria which are phagocytosed will find themselves in this acid reac-
tion, this may be an important mechanism for the destruction of bac-
teria during inflammation. Mackie, Finklestein and van Rooyen [25] have
shown, moreover, that when bacterial toxins act on leukocytes, phago-
cytic activity may be destroyed without affecting bactericidal action; in
fact, toxins may liberate the bactericidal substances from leukocytes.
It is therefore tempting to conclude that conditions which favor peptic
digestion by leukocytes also favor liberation of their bactericidal anti-
bodies. This, however, is a subject for further investigation.

SUMMARY

Whereas monocytes of rabbits contain only one proteinase, pepsin,
which is active from p_H 2 to 5, the optimal activity being shown at 3, the
polymorphonuclears have pepsin, cathepsin and trypsin with optimal
activity at p_H 3, 5.4 and 8, respectively. The serous portions of the
exudates also differ in that the monocytic type contains a substance
inhibiting peptic digestion by the leukocytes, while the polymorpho-
nuclear type enhances this activity. The fluid of a polymorphonuclear
exudate inhibits the tryptic activity of the corresponding cells, while cells
of a monocytic exudate inhibit the tryptic activity of their fluid.

There is also an inhibitory mechanism which concerns the leuko-
cytes themselves, observed by Willstätter, Bamann and Rohdewald, who
showed the presence of mutually antagonistic extractable enzyme, or lyo-
enzyme, and bound enzyme, or desmo-enzyme, in the leukocytes. This
has been confirmed, and their presence in both monocytes and poly-
morphonuclear cells of inflammatory exudates has been demonstrated.

When gelatin was digested by the supernatant fluid of either a
polymorphonuclear or a monocytic exudate, there was a decrease in
carboxylic groups, as evidenced by negative values between p_H 4.5 and
5.5 (or 6) on titration in the presence of formaldehyde. A similar
phenomenon was observed during the digestion of a dipeptide, leucyl-
glycine, by the fluid of the monocytic type. In view of the work of

27. Rous, P.: J. Exper. Med. **41**:379 and 403, 1925.
28. Jacobs, M. H.: Am. J. Physiol. **53**:457, 1920.

several authors, this is suggestive of a resynthesis of the protein-split products present in the exudate due to a reversal of catheptic action. Whether a similar process may, under proper conditions, go on in vivo during an inflammatory process is under investigation. Should this phenomenon occur, it might help to explain some phases of the problem of delayed resolution in pneumonia.

A delay in separation of an exudate into its constituents causes an inactivation of the trypsin inhibitor or a release or an activation of trypsin. It also makes conditions unfavorable for resynthesis. Gay and Clark showed that an exudate fluid loses its bactericidal power if it stays in contact with its cells for several hours. The question arises: Do conditions which decrease bactericidal power also favor resynthesis in an inflammatory exudate, and hence delay resolution? This remains to be determined.

DUPLICATION OF MECKEL'S DIVERTICULUM WITH OTHER CONGENITAL ANOMALIES

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Fitz [1] did not find a single case of duplication of Meckel's diverticulum reported in the literature prior to 1884, and I have not been able to find any since that date.

Meckel's diverticulum varies considerably in size, shape and situation in the ileum. The usual length varies from about 2 to 10 cm., but larger ones, for example, that in the classic case reported by Moll,[2] have been seen. Tubular, conical, bulbous, irregular, hammer-shaped and even lobulated diverticula have been described. Cullen [3] referred to a multilobulated diverticulum which was reported on by King in 1843 and to another diverticulum recorded by Gazin. The second one had two orifices, separated by a bridge of tissue, which opened into the intestine. Fitz mentioned the case reported by Hyrth in which the tip of a Meckel's diverticulum was divided into five parts. He described a diverticulum which had an incipient bifurcation of the tip. Nauwerck [4] noted two diverticula of the ileum; he considered one a true Meckel's diverticulum and the other an acquired diverticulum which was produced by traction by a large mass of accessory pancreatic tissue.

Meckel's diverticulum is distinguished from an acquired diverticulum by the fact that it possesses the four coats of intestine similar to those of the adjacent portion of the ileum, that is, serosa, muscularis, submucosa and mucosa.

Frequently, a Meckel's diverticulum has a mesentery or a remnant of mesentery which extends along one side of the diverticulum for a variable distance.

Christie,[5] Schaetz [6] and other investigators noted that Meckel's diverticulum is frequently associated with other congenital anomalies. Christie observed this to be true in thirty-one of sixty-three cases.

Both the diverticula I am describing in this report were observed in the terminal portion of the ileum; they were antimesenteric in position,

1. Fitz, R. H.: Am. J. M. Sc. **88**:30, 1884.

2. Moll, H. H.: Brit. J. Surg. **14**:176, 1926.

3. Cullen, T. S.: Embryology, Anatomy, and Diseases of the Umbilicus, Together with Disease of the Urachus, Philadelphia, W. B. Saunders Company, 1916.

4. Nauwerck, C.: Beitr. z. path. Anat. u. z. allg. Path. **12**:29, 1892.

5. Christie, Amos: Am. J. Dis. Child. **42**:544, 1931.

6. Schaetz, Georg: Beitr. z. path. Anat. u. z. allg. Path. **74**:115, 1925.

possessed the same coats as the adjacent portion of the ileum and were
associated with other congenital anomalies, and each had a mesentery
of its own, which extended to the tip.

REPORT OF A CASE

A hydrocephalic male fetus, 38 weeks of age, which had died in utero, was
removed by craniotomy and extraction. A diverticulum, 2.5 cm. in length and
1 cm. in width, was situated 50 cm. from the ileocecal valve on the antimesenteric
portion of the ileum. A mesentery extended to the tip along one side of the
diverticulum. Twenty centimeters proximal to this diverticulum was another,
which also was situated on the antimesenteric border and was of the same length
and width. It had a mesentery similar to that of the first. The diverticula are
shown in the figure. Microscopic sections from the base, middle portion and
tip of each diverticulum showed that they were similar to each other and to the
adjacent portion of the ileum.

In addition to the diverticula that are described there were hydrocephalus,
which was graded 3 on a basis of 4; patent ductus arteriosus with coarctation of
the aorta, and nonlobulation of both lungs.
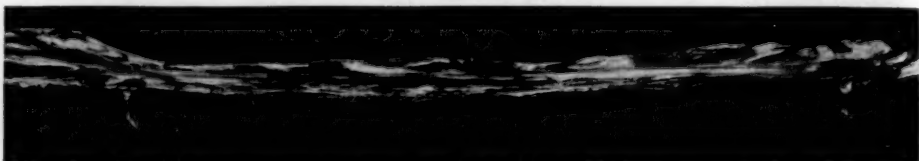
Terminal portion of the ileum, showing the two diverticula.

COMMENT

It is difficult to explain the production of two Meckel's diverticula
from the omphalomesenteric duct. I reasoned that in this case the
process of atrophy of the omphalomesenteric duct was both altered
and delayed, resulting in bifurcation of the tip similar to that which
occurred in the case reported by Fitz. This bifurcation continued from
the tip of the partially involuted omphalomesenteric duct to the base,
with the result that the duct was divided neatly into two parts, each
of which has a separate opening into the intestine. With the further
growth of the intestinal tract each diverticulum grew independently
and separated from its fellow by a distance equal to the increase in
length of the intervening portion of intestine.

SUMMARY

A case is presented of duplication of Meckel's diverticulum in a 38
week old male hydrocephalic fetus. This condition was associated with
anomalies of the nervous, cardiovascular and respiratory systems. A
possible explanation of this anomaly has been offered.

Case Reports

THROMBOCYTOPENIC PURPURA ASSOCIATED WITH ADENOCARCINOMA OF THE STOMACH IN A YOUNG ADULT

GEORGE G. STEBBINS, M.D.

AND

MARIE L. CARNS, M.D.

MADISON, WIS.

Lawrence and Mahoney [1] recently reviewed the literature on the association of carcinoma with thrombocytopenic purpura and reported one case of carcinoma of the stomach with extensive metastases and thrombocytopenia. They pointed out that carcinoma is practically always associated with a normal or an increased number of platelets in the peripheral blood and that the association of thrombocytopenia with carcinoma of the stomach is particularly uncommon. Because of the rareness of the finding of thrombocytopenic purpura associated with carcinoma of the stomach, especially in so young a person, the following case is reported.

REPORT OF A CASE

History.—A woman, aged 21, was admitted to the hospital on July 3, 1933, with a chief complaint of "pain in the legs." She dated the onset of her illness to six weeks before, at which time she had intermittent sharp stabbing pain in her shoulders. A week before her admission the pain in the shoulders subsided, but she became nauseated and weak and vomited small amounts of dark blood. The following day the stools were black, and they had continued to be so until her admission. She had had no abdominal pain at any time. Two days before admission she first noted the "pain in the legs," which was her chief complaint. This pain she localized chiefly to the regions of the hip joints. The day before admission she noted ecchymotic areas on her arms and legs. Inventory by systems contributed little more of a pertinent nature. Her maximum weight was 140 pounds (63.5 Kg.), nine years before; her average weight in recent years had been 135 pounds (61.2 Kg.), and her present weight was 120 pounds (54.4 Kg.). She specifically denied any additional symptoms referable to the gastro-intestinal tract other than recent anorexia and chronic constipation. Her past medical history showed that she had had only two diseases, measles and influenza. Tonsillectomy

1. Lawrence, J. S., and Mahoney, E. B.: Am. J. Path. **10**:383, 1934.

in 1928, appendectomy in 1929 and childbirth in 1931 had been characterized by uneventful recoveries. There was no familial history of hemorrhagic disease.

Examination.—There were marked pallor of the skin and mucous membranes, rapid regular pulse and several small areas of subcutaneous hemorrhage over the lower extremities. There was no bleeding from the mucous membranes of the nose, mouth or throat. The spleen was not palpable. There were no abnormal cardiac findings other than tachycardia (rate, 110) and no abnormal objective findings in the region of the hip joints. The impression on admission was that the condition was purpura haemorrhagica (the type to be determined by laboratory studies).

The blood count on admission showed 50 per cent hemoglobin, 2,630,000 red cells and 11,600 white cells, with 83 per cent neutrophils, 3 per cent eosinophils and 14 per cent lymphocytes. The platelet count (direct method) was 30,000; the coagulation time (capillary pipet method), five minutes, and the bleeding time, three hours (plus). A flat roentgenogram of the pelvis showed nothing abnormal in the hip joints or pelvic bones.

Course.—The patient appeared very ill and weak and complained so bitterly of pain in the hips on movement that only those laboratory studies deemed essential were carried out. Her course was progressively unfavorable despite all medication, including blood transfusions (citrated blood, from 200 to 500 cc.) of which she had a total of eighteen. Small hemorrhagic areas appeared on the buccal mucous membranes, and she expectorated small amounts of bright red blood at intervals. Terminally she showed bilateral retinal hemorrhages and evidences of intracranial hemorrhage. The average level of the platelets during her hospitalization was 80,000. The terminal blood count (reported post mortem) showed 30 per cent hemoglobin, 1,410,000 red cells and 7,950 white cells with 35 per cent neutrophils, 2 per cent eosinophils and 63 per cent lymphocytes. No abnormal cells were reported. She died on August 8, thirty-five days after admission. The final clinical impression was that the condition was primary thrombocytopenic purpura.

Summary of Autopsy.—Macroscopic Examination: Autopsy two hours after death revealed a poorly developed but fairly well nourished young white woman. The lips, gums and buccal membranes were very pale. A brownish area in the left conjunctiva was interpreted as a petechial hemorrhage. There were numerous purple petechiae on the upper extremities. A few scattered petechiae were found on the medial surface of each thigh and only an occasional one on the chest and abdomen. An oblique appendectomy scar was present in the right lower quadrant.

The peritoneal cavity contained 200 cc. of a slightly blood-tinged fluid. The peritoneal surfaces were smooth, moist and glistening. There were no adhesions. (See the paragraph on the lymph nodes.)

The thorax contained no free fluid. There were no adhesions. The pericardial cavity contained 60 cc. of a clear straw-colored fluid. The surfaces were smooth and moist.

The heart weighed 220 Gm. There were no abnormalities of the valves, endocardium, myocardium or coronary arteries. An occasional small petechia was noted in the epicardium.

The lungs were pale, voluminous and crepitant throughout. There were no consolidations or hemorrhages. The bronchi were clear. The blood vessels of the hilus contained no thrombi. The bronchial and mediastinal lymph nodes were enlarged and appeared to be producing pressure on the bronchi and blood vessels of the hilus. (See the paragraph on the lymph nodes.)

The spleen was enlarged, weighing 350 Gm. The capsule and cut surface were dark purple. The pulp was firm. The malpighian corpuscles were just visible.

The liver was enlarged and weighed 2,470 Gm. There were about a dozen small white nodules on the capsular surface up to 0.5 cm. in diameter. Sectioning of the organ revealed more nodules of the same size. Congestion was noted around some of the nodules. The cut surface of the nodules appeared white and medullary.

The wall of the gallbladder was edematous and somewhat thickened. The organ was filled with bile. Numerous petechial hemorrhages were noted on the mucosal surface. The bile ducts were patent, but somewhat compressed by enlarged lymph nodes.

The pancreas and adrenals were of normal size and appearance.

The mucosa of the stomach was uniformly dark and congested in appearance. On the lesser curvature there was a round, slightly depressed and puckered area which measured 1.8 cm. in diameter. The edges were not indurated, and there was little thickening in the base. The lesion appeared grossly like a healed peptic ulcer scar. The remainder of the alimentary tract was normal. The appendix had been removed.

The kidneys weighed 130 and 190 Gm. each. When the capsule was stripped off an occasional pitted scar could be seen on the cortex. The cortex on the cut surface measured 7 mm., and it appeared somewhat opaque and cloudy.

The ureters, bladder, uterus and adnexa were of normal appearance. The aorta was smooth and elastic.

The bone marrow of the ribs and vertebral column appeared pale and rather dry.

Examination of the lymph nodes showed: The mediastinal and tracheobronchial lymph nodes were enlarged, firm and discrete. They ranged in size up to 2.5 cm. in diameter. The nodes surrounded and appeared to be producing some external pressure on the trachea, bronchi and blood vessels of the hilus. There were similar nodes around the head of the pancreas, at the neck of the gallbladder, below the stomach and in the preaortic region. These nodes were all firm and discrete and ranged in size up to 4 cm. in diameter. Some obstruction was produced about the neck of the gallbladder by the tumor nodules. The cut surface of some of the nodules, either mediastinal or abdominal, was medullary and hyperplastic in appearance, whereas others had areas of softening and yellow necrosis. Many of the larger nodes were soft and bloody in their central portions. No noticeable extensions were found to the pelvic bones or lumbar portion of the spine. However, the eighth thoracic vertebra was the site of a moderate-sized metastasis. The ribs and sternum revealed no evidence of metastatic growths.

Microscopic Examination: The heart, the adrenals, the urinary bladder and the uterus did not show any structural changes.

The alveolar walls of the lung were slightly thickened, and there was beginning smooth muscle hypertrophy. An occasional alveolus contained a clump of red blood cells. A most interesting feature was the distribution of the tumor cells in the blood vessels and lymphatics. The peribronchial lymphatic channels were distended and filled with tumor cells (fig. 1). Other lymphatics were similarly filled, particularly those in the pleural region. Many of the blood vessels in the alveolar wall were filled with the tumor cells.

The pulp of the spleen was congested. The malpighian corpuscles were normal. There were primitive myeloid cells in the venous sinuses as well as occasional atypical tumor-like cells.

In the liver there were metastatic nodules of tumor growth. The adjacent liver cells were considerably shrunken and somewhat irregular in appearance. The Kupffer cells contained hemosiderin. Numerous radicals of the portal vein and the sinus spaces in many areas were filled with tumor cells. There were a number of foci of primitive-looking blood cells in some of the sinuses. One poorly-formed megalokaryocyte was noted.

The wall of the gallbladder was edematous.

Some of the islands of Langerhans were hypertrophied, and others appeared atrophic.

Fig. 1.—Photomicrograph of tissue from the lung. A bronchus is shown in the center, and the peribronchial lymphatic channels are distended with tumor cells.

The wall of the stomach at the site of the previously described puckered scar revealed a primary adenocarcinoma which was somewhat scirrhous in some areas. The glandular epithelium at the edge of the invasive growth was undergoing malignant degeneration. Lymphatics and smooth muscle in the deeper portions of the section were all being invaded. There was little connective tissue response. Many mitotic figures were noted (fig. 2 B).

The kidney tubules were somewhat swollen and granular. There were subcortical collections of lymphoid cells, and the subcortical glomeruli were becoming obliterated and hyalinized.

The lymphatics in the ovary were filled with tumor cells, but no perilymphatic invasion had occurred. There were areas of necrosis and hyalinization.

The lymph nodes were largely composed of tumor cells which were actively growing. There were small areas of necrosis and hemorrhage. Many mitotic figures were noted.

The bone marrow of the rib had an exhausted sclerotic appearance. The hematopoietic centers were appreciably reduced in numbers. There were no megalokaryocytes. A considerable amount of hemorrhage had occurred. Recent hemorrhages as well as areas of organizing hemorrhage were apparent. The main picture was that of sclerosis of the marrow. These scars were interpreted as areas of previous hemorrhage which had since become organized and replaced by connective tissue.
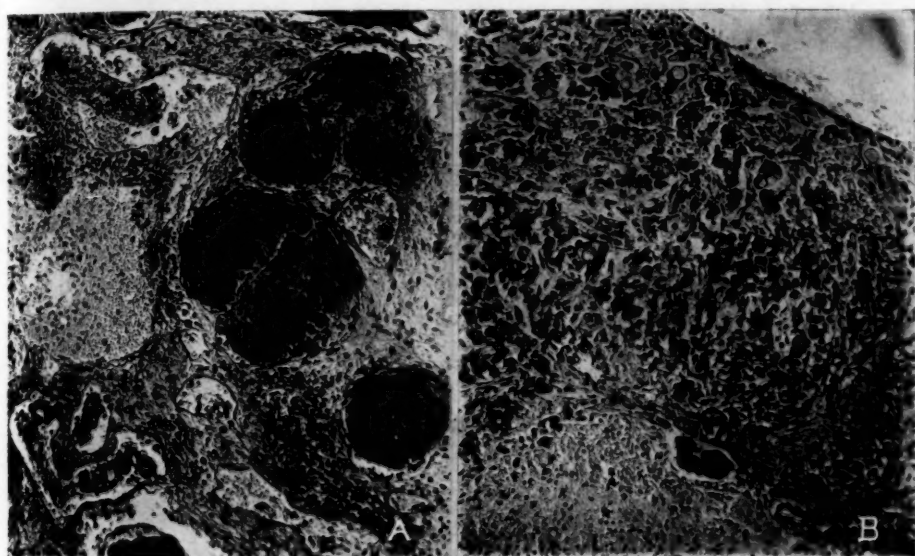
Fig. 2.—*A*, photomicrograph of bone marrow of the rib which reveals markedly reduced hematopoietic centers, hyaline thrombi on the right, areas of hemorrhage and venous sinuses in the left lower corner filled with tumor cells. *B*, photomicrograph showing primary adenocarcinoma of the stomach; tumor cells are seen in the lymphatics.

The arterial capillaries stood open and contained only red cells, but the thin-walled venous sinuses were filled with tumor cells. It seemed probable that behind the blocked sinusoids hemorrhages had occurred. Many of the sinusoids were filled with thrombi, some of which were becoming hyalinized. Only an occasional tumor cell was found outside the sinuses (fig. 2*A*).

Sections from the vertebral metastasis revealed these pictures: One had tumor cells filling most of the sinuses and only an occasional small extravascular extension; another area revealed numerous patches of connective tissue scars. The major growth of tumor cells was in the sinuses.

SUMMARY

An interesting feature of this case was the predilection of the tumor cells for the lymphatics, lymph nodes and venous sinuses. The cells from the small primary gastric carcinoma readily invaded the lymphatics and gave rise to the large number of metastases to the lymph nodes noted in the mediastinum and peritoneal cavity. Lymphatic involvement in organs was particularly noted in the ovaries and lungs. Not a metastasis was found in the lungs outside the pulmonary lymphatics, which were widely distended, especially in the peribronchial regions. The liver had a number of metastatic tumor nodules, and in addition numerous radicals of the portal vein and liver sinusoids were filled with tumor cells.

The venous sinuses of the bone marrow were filled with the tumor cells. It is our belief that the marked invasion and proliferation in these sinuses played a leading rôle in the changes produced in the peripheral blood picture. The exhausted appearance of the marrow with the marked reduction of hematopoietic centers and the apparent absence of megalokaryocytes would account for the reduction in hemoglobin and red blood cells as well as the diminution in the number of platelets. It is of interest in this connection that in the similar case reported by Lawrence and Mahoney[1] the marrow showed an approximately normal number of morphologically normal megalokaryocytes.

SPIROCHETAL ABSCESS OF THE LIVER

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Although spirochetes and fusiform bacilli and other associated organisms are commonly found in infections of the mouth (such as fusospirochetal angina, noma, pyorrhea alveolaris and periapical infections), in infections of the genital organs, intestinal tract and respiratory tract and, less commonly, in those of the eye, ear, nose, meninges and brain, their occurrence in abscess of the liver, osteomyelitis of the cranial bones and lesions of the axillary glands is rare.

In 1926 Von Glahn [1] reported that he had found argentophilic microorganisms in two cases of supposedly sterile hepatic abscesses. The demonstration was made by the silver impregnation method of Levaditi. The spirilla observed in the first case stained intensely. They varied in form: some were long and undulating, containing at times as many as nine half convolutions; others were not so definitely convoluted but were curved and slightly twisted. The ends were rounded. In the second case, large masses of long, slender bacilli were seen. Many of these organisms were sharply curved, others were slightly curved, but none had convolutions similar to those described in the first case. No dark-field examinations were made. Zinserling [2] observed metastatic abscesses of the liver and kidney in a case of fusospirochetal infection of a carcinoma of the stomach, and Smith [3] observed metastatic abscesses of the liver, kidney and spleen and also a metastatic fusospirochetal abscess of the femur following an operation on a fusospirochetal abscess of the lungs. Considering that hepatic abscesses have been described as sterile in about one half or more of the cases reported (Rolleston,[4] Talbot,[5] Elsberg [6] and Giordano [7]), we agree with Von Glahn that more careful bacteriologic examinations should be made in cases in which no bacteria or actinomycetes appear to be present in the abscesses.

REPORT OF A CASE

A white man, aged 40, was admitted to the Lakeside Hospital on June 9, 1931, complaining of pain in the chest and swelling of joints of the fingers, wrists, knees

1. Von Glahn, W. C.: Proc. New York Path. Soc. **26**:97, 1926.

2. Zinserling, W. D.: Ueber die fuso-spirochätose Gangrän und einige Prozesse vorzugsweise bei Kinder, Jena, Gustav Fischer, 1928.

3. Smith, D. T.: Oral Spirochetes and Related Organisms in Fuso-Spirochetal Disease, Baltimore, Williams & Wilkins Company, 1932.

4. Rolleston, H. D.: Diseases of the Liver, Gall-Bladder and Bile-Ducts, New York, The MacMillan Company, 1912.

5. Talbot, P.: Brit. M. J. **2**:375, 1919.

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7. Giordano, quoted from Davidsohn, C.: Virchows Arch. f. path. Anat. **171**:523, 1903.

and ankles. He gave a history of having had influenza followed by pneumonia, hemoptysis and the coughing up of large amounts of foul-smelling sputum. Other features of the past history and the familial history were irrelevant. At the hospital he showed marked pallor of the skin and mucous membranes. Respiration was rapid and shallow but not labored. There was a fetid odor to the breath. The teeth were in poor condition, and the gums were retracted. The head, ears and nose were normal. The right side of the chest was flattened anteriorly, with a posterior bulge beneath the scapula. The respiratory excursion on the right was somewhat restricted, and dulness to percussion at the base of the right lung with elevation of the breath sounds was noted. Râles occurred at the end of inspiration. Just beneath the angle of the scapula on the right, amphoric breathing was heard. The heart was not enlarged, but the beats were rapid, 110 per minute, and of fair quality. No murmurs were heard. The abdomen and genitalia were normal. There was clubbing of the fingers. The joints and the wrists, fingers, elbows, knees and ankles were swollen, with increased local heat and redness. The white blood cell count was 13,000; the erythrocyte count, 3,600,000, and the hemoglobin, 70 per cent. The Wassermann reaction was negative. The sputum showed a mixed flora but no spirochetes or acid-fast forms. The patient remained in the medical service seventy-four days, and during this time his sputum showed spirochetes and fusiform bacilli. Postural drainage was maintained, and five injections of neoarsphenamine were administered. Bronchoscopy showed nothing unusual. A rib resection was performed on the level of the angle of the scapula, and the abscess was drained. Following irrigation of the wound with surgical solution of chlorinated soda and eight days prior to death, a positive blood culture (Streptococcus gamma) developed and signs of a fibrinous pleurisy appeared at the base of the left lung.

The clinical diagnosis was: abscess of the middle lobe of the right lung, bronchopneumonia, fibrous pleurisy and septicemia (Str. gamma).

Autopsy.—Only the significant observations in the lungs and liver will be dealt with.

The cut surfaces of the upper lobe of the left lung revealed two adjacent spherical cavities near the periphery, measuring 1.5 by 2 cm. The walls of the cavities were thin, soft and structureless. They contained a dark green material, and no direct communication with the respiratory passages was observed. The overlying pleura was thickened.

The right lung appeared larger than normal and showed a considerable increase in weight. The pleural surface over the middle lobe, over the upper portion of the lower lobe and over the lower portion of the upper lobe appeared thickened, gray and fibrous, and showed numerous fibrous tags and bands. Cut surfaces revealed the middle lobe to be of greatly increased density, to contain a spherical cavity about 2.5 cm. in diameter, and to have a fibrous wall from 2 to 3 mm. in thickness. The cavity contained a thick, yellow, semifluid material, and its lining appeared to be structureless. The cut surface immediately surrounding this cavity showed increased density, appeared gray and fibrous and contained a small group of irregular areas of softening, varying in width up to 4 mm. These areas were in direct communication with the sinus in which the rubber drainage tube was inserted.

There was no communication between the thoracic and abdominal cavities.

On the superior aspect of the right lobe of the liver, at about the middle portion, the capsule was thinned out and showed a circular area (cavity) of dark green color, approximately 8 cm. in diameter. When this was opened, a thick,

yellow fluid having a foul odor escaped. The wall of the cavity was 4 mm. in thickness, dark gray and soft.

Pathologic Diagnosis.—Gangrenous bronchiectasis of the right lung and gangrene of the liver; chronic interstitial pneumonia; the wound of a surgical incision of the right thoracic wall with a sinus leading to the middle lobe of the right lung; acute fibroserous pleuritis of the left side; chronic fibrous pleuritis of the right side; acute fibrous pericarditis; acute hyperplasia of the spleen; cloudy swelling and passive hyperemia of the kidneys.

Bacteriologic Examination.—Pus from the left lung contained fusiform bacilli, spirochetes, rods, cocci and pus cells. Some of the spirochetes observed by dark-field illumination and Fontana's stain appeared to be large and wavy (Spirochaeta buccalis) and others finer, wavy or closely coiled (Spirochaeta Vincenti). In the hemorrhagic exudate from the right lung masses of spirochetes of the same types were seen, in addition to fusiform bacilli and other bacilli of various sizes, cocci and pus cells.

The pus from the liver was yellow and showed masses of pus cells, vibrios and cocci. No spirochetes, typical fusiform bacilli or cysts were seen. The cavity of the liver was washed out with physiologic solution of sodium chloride, and a piece of hepatic tissue at the base of the ulcer was taken out, ground up and examined by dark-field illumination, and smears stained by Fontana's and Howell's [8] methods. By these methods the spirochetes predominated.

The spirochetes were fine, and the majority appeared to be well coiled, showing an average of from seven to ten coils. Some were closely coiled, others loosely coiled. By dark-field illumination they appeared to be actively motile. The more closely coiled forms gave the appearance of Treponema dentium, although the majority appeared to be of the type of S. Vincenti. In the hepatic tissue stained by Fontana's method, they appeared in the intercellular spaces. Cultures on numerous mediums containing serum, ascitic fluid or hydrocele fluid and inoculations into traumatized testicles of rabbits failed to propagate them. The blood culture yielded Str. gamma.

SUMMARY

A case of spirochetal gangrene of the lungs and liver is described. The organisms in the hepatic tissue were discovered by dark-field examination of material from the base of the hepatic abscess, and the observation was confirmed when the hepatic tissue was stained by Fontana's method. No direct connection existed between the abscess of the liver and the abscesses of the lungs. The abscess of the lung was therefore considered to be of metastatic origin.

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8. Howell's method of staining employs 1 per cent potassium permanganate and methyl violet 6 B.

Laboratory Methods and Technical Notes

DI-NITROSORESORCINOL—A NEW SPECIFIC STAIN FOR IRON IN TISSUES

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The demonstration of compounds of iron, such as hemosiderin and various other allied iron compounds in tissues, has changed but little in technic in the last seventy years, and at present various modifications of the prussian blue reaction are employed almost to the exclusion of other methods. In the course of work on the recognition of iron in various organs it was found that all the present methods are wanting in one or more respects, and a search was made for new specific stains for iron. Many of these methods, such as the use of a sulphide, lack specificity and fail to distinguish iron pigments from coal dust, silver salts and other dark materials and yet are superior to the specific ferrocyanide reactions which are relatively much less sensitive. This defect in sensitivity was conclusively demonstrated by testing the various methods on serial sections of iron-containing tissue and noting the variation in the amount of demonstrable pigment. The impermanent nature of the prussian blue reaction is also an objection, because in experimental work over a period of years marked fading was noted in slides less than two years old.

In an effort to find a more satisfactory stain for iron various reagents which gave a specific color reaction in colorimetric and metallurgic chemical analyses were used on iron pigment-containing tissue. Alcoholic and aqueous solutions of these chemicals were tested for their reactions to iron on sections from tissues fixed in formaldehyde and mounted in paraffin. In some instances the sections were first sensitized by a quick immersion in a solution of ammonium sulphide. The following specific reagents for the detection of iron were used: acetylacetone, an alcoholic solution of di-methylglyoxime, ammonium phenylnitrosohydroxylamine, alloxantin, thioglycollic acid and di-nitrosoresorcinol. The last mentioned compound was found to be the only satisfactory stain. Its formula after drying, according to Orndorff and Nichols,[1] is $C_6H_2O_2(NOH)_2H_2$; when it occurs as 2, 4, di-nitroresorcinol, the formula is $(C_6H_2 (N)_2)_2 (CH)_2 2H_2O$, according to Rosenthaler.[2] It is a dark brown powder, or it may occur as yellow-brown lamellae

1. Orndorff, W. R., and Nichols, M. L.: J. Am. Chem. Soc. 45:1536, 1923.

2. Rosenthaler, L., in Margosches, B. M.: Die chemische Analyse, Stuttgart, Ferdinand Enke, 1923, 1920, p. 483.

which detonate at 115 C., according to Meyer and Jacobsen,[3] or at 142 C., according to Rosenthaler. Fitz,[4] in 1875, first mentioned the formation of a green coloration or lake with ferric chloride. Di-nitrosoresorcinol is soluble with difficulty in cold water and alcohol, but is soluble in ether, benzene, toluene and chloroform. For the detection of iron the solution should be neutral.

It was noted in the work with this stain that if the tissue was first sensitized by a mere dip in ammonium sulphide the stain was much more satisfactory and the iron stained rich green; moreover, if the slide was allowed to remain in the solution a few hours or overnight a pleasing brown counterstain resulted. The delicacy of this reaction is probably due to the ability of the new reagent to render minute quantities of iron visible, for it is evident that the sulphide renders the iron sensitive for the characteristic color reaction with the specific reagent. Potassium cyanide can be employed as a sensitizer, but it is not as satisfactory as the sulphide.

The reagent appears to be more effective after it has aged for a few days; this is possibly due to its relative insolubility. It seemed feasible to maintain a layer of the undissolved reagent in the bottom of the jar to insure saturation. The solution remains apparently unaltered and stains well after two years of almost constant use, although it becomes darker.

The tissue, after fixation in formadehyde, is blocked in paraffin, sectioned and mounted on slides; after drying and removal of the paraffin the sections are stained by the following method:

1. Place the section in a jar containing a dilute (30 per cent) solution of ammonium sulphide for one minute.

2. Rinse in water.

3. Place in a staining jar containing a saturated aqueous solution of di-nitroso-resorcinol[5] for from six to twenty hours, depending on the depth of the counter-stain desired. (If an alcoholic solution is desired use a 3 per cent concentration in 50 per cent alcohol.)

4. Wash in water or dilute alcohol.

5. Run through graded alcohols, carboxylol and xylol as usual.

6. Mount in balsam.

The sections appear brilliant, sharp dark green against a rich brown background. The cellular outline is clear, and the pigment granules do not fade to brownish red as in old slides stained with prussian blue. Slides which I stained in February 1930, over five years ago, still retain their pristine brilliance; sections stained at the same time with prussian blue from a block in the same series have a faded and unsat-

3. Meyer, V., and Jacobsen, P.: Lehrbuch der organischen Chemie, Berlin, W. de Gruyter & Co., 1902, vol. 2, p. 464.

4. Fitz: Ber. d. deutsch. chem. Gesellsch. **8**:631, 1875, quoted by Orndorff and Nichols.[1]

5. Di-nitroresorcinol used in this method was obtained from the Division of Organic Chemicals of the Eastman Kodak Co., Rochester, N. Y.

isfactory appearance. If the usual red counterstain is desired, the time given the third step in the process can be decreased to one hour or less.

The method outlined compares in specificity and sensitivity with any of the older methods, and unless speed is essential the counterstain will be found satisfactory. Except for the dip in the sensitizing sulphide, the process is reduced to one stage. The permanence of the stain, in contrast to that obtained by the contemporary methods in vogue, should warrant its use if the slides are to be kept for future reference.

General Review

EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS AND ITS RELATIONSHIP TO HUMAN ARTERIOSCLEROSIS

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VIII. ETIOLOGY AND PATHOGENESIS OF EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS IN RABBITS

The existing information concerning experimental cholesterol arteriosclerosis has been outlined in the survey of the literature presented in the preceding sections of this paper. More information on many points could be desired, and it is evident that a number of ideas which have found acceptance in certain quarters lack the substantiation of thorough experimental proof. Nevertheless, the evidence which may be regarded as reasonably well established is sufficient to serve as a basis for some deductions as to the etiology and pathogenesis of this experimental disease.

It will be recalled that it has been possible only in rabbits and guinea-pigs to show at all clearly that cholesterol feeding is capable of producing lesions in the arteries. So far as guinea-pigs are concerned, not much more than this is known. It is true that the arterial lesions which develop in guinea-pigs following the prolonged feeding of cholesterol-rich diets are similar to those found in rabbits treated in the same way, and it might be inferred from this fact that the manner of development of the lesions is practically identical in the two species of animals. However, so little is known concerning experimental cholesterol arteriosclerosis in guinea-pigs that nothing would be gained at present through an attempt to discuss the pathogenesis of the disease in these animals. Accordingly, this subject will be excluded from further consideration here, and the following discussion will be limited to the etiology and pathogenesis of experimental cholesterol arteriosclerosis in rabbits.

Before the importance of cholesterol in the diet was recognized, the earlier investigators [s] thought that the fatty arterial lesions which they had produced in rabbits were the result of a "fatty degeneration" of the arteries due to the toxic effects of the animal proteins in the diets

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[s] 47, 65, 66, 147, 148.

which they had used, and to the action of certain "mechanical" factors. Later, the production of similar lesions by the feeding of cholesterol alone or of cholesterol in oil showed that the proteins were not of essential importance, and attention was shifted to the rôle of cholesterol in the etiology and pathogenesis of the arterial lesions. It was soon realized that all of the lipoid material which appeared in the walls of the arteries could not arise from the local breakdown of tissue, and it became necessary to account for its arrival from elsewhere.

The possibility was suggested that the progressive deposition of doubly refractive lipoids in the liver, spleen, bone marrow, suprarenal cortex and other tissues and finally the appearance of lipoids in the walls of the arteries represented a series of compensatory processes designed to relieve the blood stream of an excessive load of cholesterol which could not be handled adequately by the excretory apparatus.[a] This explanation has not been accepted widely because it neglects the fact that the deposits of cholesterol in the animal body occur in two distinct ways. The lipoids which appear in the cells of the suprarenal cortex and of the liver and in the cells of the reticulo-endothelial system seem to be taken up by these cells directly from the blood stream. But another kind of deposit occurs in which the lipoids are first precipitated in relation to the intercellular ground substance of tissues and may then be taken up secondarily by cells which subsequently are stirred into activity. These two processes are essentially different. The first represents a normal function which is exaggerated under the conditions of the experiments. So far as it assists in reducing the level of the blood cholesterol by temporary storage or otherwise, it may be considered as compensatory. However, the deposition of cholesterol and of other lipoids in intercellular materials is a very different process, not acting under normal conditions and not to be regarded as compensatory, but rather as definitely pathologic. It is this process which must be explained to account for the appearance of fatty deposits in the arteries of cholesterol-fed rabbits.

The theory of the pathogenesis of experimental cholesterol arteriosclerosis which has found the greatest favor is a modification of the so-called "infiltration" or "imbibition" theory. The chief proponents of this theory, as it is applied to experimental cholesterol arteriosclerosis, have been Anitschkow and his associates. Anitschkow's most recent statement of his views [12] represents the epitome of all the ideas which he had previously expressed, and consequently only this publication need be drawn on for the following résumé of the "infiltration" theory.

According to this theory, the lipoids which make their appearance in the arterial wall do not originate from "degeneration" of the tissue,

(a) 99, 100.

but infiltrate or impregnate it from the blood stream. The wall of the vessel is regarded as being perfectly normal and unimpaired in structure prior to the advent of lipoid substances. The subsequent proliferation in the intima is viewed as the result of the presence of precipitated cholesterol, the precipitation of lipoids being the primary event. The lipoids in colloidal state enter the intima from the lumen of the artery with the "nutritive lymph stream" which normally seeps through the entire thickness of the wall of the vessel and is then carried off by the lymph channels or veins of the adventitia. During this slow permeation of the arterial wall, the "lymph," which presumably carries an increased concentration of lipoids, has to penetrate the ground substance and the latter accordingly becomes impregnated with fatty substances. The cholesterol and associated lipoids undergo "flocculent precipitation" during this slow passage, and they appear in the ground substance as stainable particles. In explaining the precipitation of the lipoids, great importance is attached to the normal physicochemical properties of the ground substance and to the slowness with which the "lymph" permeates the arterial wall. The usual preponderance of lipoid deposit in the intima is accounted for by the resistance offered to the onflow of the "nutritive lymph stream" by the internal elastic lamina. The consequent slowing of the "lymph" stream is supposed to favor the precipitation of lipoids which therefore appear first and most abundantly in the intima.[a1]

(a¹) In speaking of the pathogenesis of human arteriosclerosis, Rosenthal (Arch. Path. **18**:660, 1934) recently added to the infiltration theory as here stated a further elaboration, the essential ideas of which are contained in the following quotation: "The serum of the blood stream infiltrating through the inner two thirds of the aorta may show a hindering of its lipoids at the elastic barriers. With contraction of the vessel in diastole there is an expression of this substance. . . . The lipoid deposit is dependent on the disproportion of the infiltration over expression under normal conditions. It is for this reason that muscular arteries, which have markedly developed internal elastic membranes that contract more vigorously than the other constituent parts of the vessel, express the lipoids more efficiently." In this highly theoretical statement, there is an unfortunate confusion between the infiltrating, lipoid-containing fluid, on the one hand, and the lipoids which may be precipitated from it in the form of a deposit, on the other. The fundamental question of the actual cause of the deposition or precipitation of lipoids from the colloidal state in which they exist in the infiltrating fluid is not touched on. The hypothetical failure of diastolic recoil to force infiltrating fluid out of the arterial wall could lead only to edema of the tissue, and so long as the lipoids remained in colloidal suspension they could not accumulate in one place. The precipitation of lipoids remains entirely unexplained. As to the "expression" of lipoids when once they have been precipitated in the form of a deposit, the idea that the mere mechanical pressure exerted by the diastolic contraction of any artery could remove the lipoids by forcing them back into the blood stream or back into solution in the tissue fluid seems positively fantastic.

From all of this, it is obvious that only one deviation from the normal condition is conceived as a necessary factor in the precipitation of lipoids, namely, an increased concentration of lipoids in the "nutritive lymph stream," a condition which presumably follows as a result of the hyperlipemia occasioned by cholesterol feeding. Up to this point, however, no explanation has been offered to account for the patchy distribution of the fatty deposits. If the function of the "nutritive lymph stream" is to nourish the inner layers of the wall of the vessel, it must permeate all parts of the intimal surface, and therefore lipoid deposits should occur diffusely over the whole extent of the artery. This difficulty has been obviated by the postulation of normal local differences in the permeability of the arterial walls. Support for this idea has been sought in some experimental work which may be mentioned briefly.

The conception of the normal flow of colloid-containing fluid from the lumen of the vessel into its walls is based on observations on the behavior of colloidal dyes, such as trypan blue, when they are introduced into the blood stream.[b] Shortly after trypan blue is injected, the walls of the arteries become stained. In the aorta, certain areas are stained more deeply than the remaining parts, and these areas correspond fairly well with the localities in which fatty deposits occur most frequently in experimental cholesterol arteriosclerosis. Anitschkow[12] believes that most of the dye enters the wall of the aorta from its lumen, and that the lining endothelium of the aorta is just as permeable to colloids as are the capillaries. The more deeply stained areas are interpreted as indicating localities in which the outflow of the "nutritive lymph stream" from the lumen is normally more pronounced than elsewhere. The more abundant flow of lipoid-containing "lymph" through such local areas is offered as an explanation of the corresponding localization of fatty deposits in the aortas of cholesterol-fed rabbits,[c] although such an explanation is entirely inconsistent with the conception that the slowness a flow of the centrifugal "lymph" stream is of importance in the precipitation of lipoids.

I have repeated these experiments with the staining of the arteries of rabbits by the intravenous injection of trypan blue,[44] and the results show clearly that, although the lining endothelium of the aorta is slightly permeable to the dye, it is not nearly so permeable as are the vasa vasorum. Furthermore, the permeability of the aortic lining is everywhere uniform. The more deeply stained areas seen on the intimal surface of the aorta are due to the escape of dye from the capillaries of the vasa vasorum, which have been shown by Robertson[125] to be much more abundant in these localities. The trypan blue deposited in the adventitia and outer layers of the media is visible on the intimal

(b) 10, 12, 55, 57, 83, 115, 120.
(c) 55, 57, 115.

surface because of the thinness and translucency of the rabbit's aorta. This fact apparently has led to the mistaken idea that the dye had entered from the intimal side.

These observations preclude the possibility of explaining the localized character of the lesions of experimental cholesterol arteriosclerosis on the basis of the "infiltration" theory as it is stated by Anitschkow. This fact, in turn, throws serious doubts on the adequacy of the explanation offered for the precipitation of lipoids in the arterial walls, an explanation which in itself is far from convincing. An increased concentration of lipoids in the "nutritive lymph stream" seems insufficient to account for the precipitation of lipoids in certain localized areas which show no normal differences from other comparable areas where lipoid deposits do not occur. For these and other reasons I feel that the "infiltration" theory, as it stands, does not provide a completely satisfactory explanation of the development of experimental cholesterol arteriosclerosis. I agree that the lipoids which appear in the arterial walls accumulate in such large quantities that they could not arise from local destruction of tissue. Chemical analyses to verify this point are not available, but it is so obvious that it is accepted as a fact by almost every one. It is not doubted, then, that the lipoids come from elsewhere to be deposited in the intercellular ground substance of the walls of the vessels. However, the conditions which bring about the precipitation of extraneous lipoids in the walls of arteries are not by any means clear. It seems worth while, therefore, to reconsider this aspect of the pathogenesis of experimental cholesterol arteriosclerosis.

If the lipoids which accumulate in the arterial walls come from elsewhere, they must be brought by way of the blood stream. From the experiments with colloidal dyes already mentioned, it seems clear that colloid-containing fluid derived from the blood plasma normally permeates the tissues of the wall of the vessel, partly from the lumen of the vessel and partly from the vasa vasorum. This fluid which serves the nutritional requirements of the tissues of the artery must be the immediate source of the lipoids which accumulate in the wall of the vessel. However, cholesterol and other lipoids are normally present in the rabbit's plasma, and presumably the nutritive tissue fluid contains a proportional amount of lipoids, but lipoid deposits do not occur in the arteries of rabbits fed on normal diets, not even when local injuries to the wall of a vessel are produced which are known to favor the deposition of lipoids. It follows, therefore, that some change in the character of the nutritive fluid which permeates the wall of the vessel must occur before the deposition of lipoids becomes possible.

Information as to the nature of this change in the character of the nutritive fluid should be forthcoming from the data concerning the

development of experimental cholesterol arteriosclerosis. Cholesterol feeding is the one factor which is known definitely to be essential to the production of the lipoid deposits in the arteries. The consequences of cholesterol feeding, therefore, merit first consideration. Following the ingestion of cholesterol, hypercholesteremia appears. But it is not so much dependent on an increase of free cholesterol in the blood as on an elevation of the esters of cholesterol with fatty acids; and, more than a simple hypercholesteremia, there is a general hyperlipemia. With these facts in view, there seems to be no good reason for focusing attention on the hypercholesteremia alone, especially since it is well known that the stability of cholesterol in solution depends to a great extent on the concentration of the other lipoids which are associated with it. However, almost all of the available data are concerned only with the cholesterol content of the blood, so that one is forced to refer repeatedly to hypercholesteremia as though it were the only change in the blood which is worthy of consideration. Lack of more complete information is the only reason for doing so.

The assumption has already been made that some degree of hypercholesteremia is necessary to the development of experimental cholesterol arteriosclerosis in rabbits (section VI *b*). It is not known that this is the only necessary change in the blood, but granting that hypercholesteremia is essential, a variety of ways in which it could exert an influence may be imagined. One almost certain consequence of hypercholesteremia, and of the hyperlipemia which probably always accompanies it, is an increase in the lipoid content of the nutritive fluid which permeates the walls of vessels. The nutritive fluid may be altered in other ways as well, but nothing has been sought or studied in the blood except the lipoids, so that an increased concentration of lipoids in the nutritive fluid is the only specific alteration which can be assumed with confidence. For the present, then, one must be satisfied with this as fulfilling the theoretical requirement that a change in the character of the fluid which permeates the arterial walls must occur before the deposition of lipoids become possible. In any event, it is obvious that following the feeding of sufficient quantities of cholesterol, the general conditions in the blood and secondarily in the nutritive fluid of the walls of vessels are suitable for the formation of lipoid deposits in the walls of the arteries.

Now, since the lesions of experimental cholesterol arteriosclerosis are local and not diffuse, it is self-evident that the conditions in the wall of the artery at the sites where lipoids are deposited are different from those which obtain in the parts of the same artery where no lipoid deposits occur. The conditions which exist at the sites of lipoid deposi-

tion and which do not exist elsewhere must constitute the immediate cause of the precipitation of lipoids in those areas, and it is equally obvious that these conditions must exist before any precipitation takes place. It is important to inquire into the nature of these local conditions in the arterial wall which are responsible for the precipitation of lipoids from the colloidal state in which they enter the wall of the vessel with the nutritive fluid. For the sake of convenience these conditions will be referred to as the "local precipitating conditions."

It is conceivable that the local precipitating conditions exist normally in normal arteries, but this seems most improbable. The idea that the permeability of the endothelial lining of the aorta is normally greater in certain areas than in others is based on what seems to be a misinterpretation of the results of experiments with colloidal dyes. Careful repetition of these experiments [44] indicates that the lining of the aorta, as might be expected, is everywhere equally permeable to the dye. Indeed, it is difficult to imagine the existence of local precipitating conditions in normal arteries, especially since these conditions must exist only in certain local areas and not in others. In the aorta, for example, one would have to assume that the local precipitating conditions were present normally in certain areas, while in adjacent comparable areas they were not. If only the regions around the mouths of branching arteries became the sites of lipoid deposits, one could believe that the unique conditions existing there might be sufficient to determine the deposition of lipoids in those areas and not elsewhere, but actually, localized lipoid deposits can develop almost anywhere in the aorta and the pattern of distribution of the lesions is never twice exactly the same. In fact, the progressive appearance of increasing numbers of localized lesions in the arteries during the course of cholesterol feeding experiments gives clear indication that the local precipitating conditions in the walls of the vessels develop progressively after the experiment has been commenced. The appearance of the first patch of lipoid deposit shows that the general conditions in the blood, etc., are suitable for the precipitation of lipoids in the arterial walls, and yet the other localized fatty lesions which develop subsequently make their appearance one by one as the experiment continues, deposition of lipoids commencing in each new area only after the appropriate local precipitating conditions have been established.

These partly theoretical considerations are verified by the actual observation of histologic changes in the arterial walls which develop during the course of cholesterol feeding experiments and which evidently precede the deposition of lipoids. In the intima, the ground substance becomes swollen so that the subendothelial layer is distinctly thicker than usual. The preliminary changes in the media are more conspicuous, consisting of focal necrosis and dissolution of muscle fibers

so that only a rather cloudy or flocculent ground substance remains. In both the intima and the media the altered ground substance subsequently becomes impregnated with stainable lipoid particles. The preliminary histologic changes are apparently the visible expression of the development of the local conditions in the arterial wall which constitute the immediate cause of the precipitation of lipoids. It is impossible to say what invisible changes in conditions may accompany the visible alterations and consequently the exact manner in which the precipitation of lipoids is brought about cannot be specified. It is evident that the intercellular ground substance is altered in some way. Probably there is a change in its physicochemical state, a change perhaps akin to coagulation. Whether this alteration in itself is sufficient to explain the results is not known; it seems that it may well be. In any case, the sum total of the local change in conditions confers on the altered ground substance the property of accumulating precipitated lipoids from the nutritive fluid which permeates the walls of the vessels.

It seems evident that the preliminary local changes in the arterial walls are due to some kind of injury. This is especially obvious in the initial alterations in the media where there is actual necrosis of muscle fibers. It can be inferred from this that the associated changes in the intima are likewise due to injury. This conclusion receives the strongest sort of support from the evidence advanced in section VI f of this paper, and it is entirely consistent with every known fact concerning experimental cholesterol arteriosclerosis in rabbits. However, it is impossible at present to specify the cause of the arterial injury. This should be in no way surprising since the rôle of the primary injury to the arteries has never been emphasized previously, and the problem of its cause remains practically untouched. Evidently some unsuspected factor connected with the feeding of cholesterol is injurious to the rabbit's arteries, which are well known to be extremely susceptible to noxious agents of many kinds. I have pointed out that the preparations of supposedly pure cholesterol which have been used in the past were probably not composed of a single sterol [119] but almost certainly contained ergosterol and possibly other sterols, which might well have a damaging effect on the arteries of rabbits even though present in the diet in relatively small quantities. Perhaps the feeding of large amounts of cholesterol and oil entails some other departure from a normal adequate diet. Possibly the hyperlipemia, which is often excessive, upsets the general metabolism sufficiently to effect an injury to the arteries. Danisch [37] suggested that nervous influences are of importance, but the cause of the arterial injury is really quite unknown and urgently requires investigation. However, this problem being left unsolved for the present, the fact remains that a primary injury to the arterial walls occurs and plays an essential part in the development of experimental cholesterol arteriosclerosis.

From all that has been said, it seems clear that the occurrence of localized lipoid deposits in the walls of arteries is dependent on the coexistence of two sets of conditions. One set includes the conditions which must obtain in the blood and secondarily in the nutritive fluid which permeates the walls of the vessels. These may be called the general conditions. The second set comprises those conditions which must exist locally in the walls of the arteries at the sites where lipoid deposits are to occur. These may be called the local conditions. When both the local and the general conditions are suitable, lipoid deposits will develop. It seems probable that neither set of conditions exists normally in the rabbit. It has been assumed that hypercholesteremia with a consequent increase in the lipoid content of the nutritive fluid is one of the necessary general conditions. In any event, no other general conditions have been recognized. The appropriate local conditions follow as the result of some sort of injury to the arterial walls. An alteration in the state of the intercellular ground substance of the vessel wall, due to the arterial injury, can be regarded as one of the necessary local conditions. No other local conditions have been recognized specifically.

Since both of the recognized factors—hypercholesteremia and injury to the arteries—are conceived as being necessary to the development of experimental cholesterol arteriosclerosis in rabbits, it follows that either factor existing alone will be ineffective in producing the fatty lesions in the arteries. It is well known that the lesions produced in the arteries of rabbits by various kinds of injury (e. g., cauterization, injections of epinephrine or nicotine, etc.) always remain practically free from stainable fat so long as the blood lipoids are at normal levels. It is also clear that hypercholesteremia of marked degree can follow cholesterol feeding in rabbits without producing any lesions in the arteries. This fact has been pointed out already in section VI *b* of this paper. Moreover, it has been shown in section VI *f* that the general conditions in the blood may be suitable for the development of lipoid deposits in the arterial walls without the occurrence of any such deposits in normal parts of the arteries. In the same place it was pointed out that the most abundant accumulations of lipoids develop when the feeding of cholesterol is supplemented by other procedures which injure the arteries more rapidly and severely. All of these facts confirm the general conceptions set forth in the preceding paragraph.

When the precipitation of lipoids in the arterial walls has been accounted for, the further development of the lesions of experimental cholesterol arteriosclerosis follows naturally enough. The increasing abundance of the lipoids in the arterial lesions evidently depends on the continued action of the factors which are responsible for the initiation of lipoid deposition. Evidence of the continuation of injury to the arteries can be found in the progressive destruction of muscle and

elastic tissue in the inner layers of the media. In the intima, however, all else is masked by the density of the lipoid deposits and by the cellular proliferation which follows. The proliferative response is generally regarded as a direct result of the presence of precipitated lipoids in the intima, special emphasis being laid on the presence of cholesterol esters. Such a relationship is obvious in the case of the macrophages which appear in the intima and take up the lipoids with such avidity. It seems probable, too, that the presence of lipoids can stimulate the proliferation of fibrous connective tissue cells, but the effect of the primary injury in producing a reparative reaction cannot be disregarded. Nevertheless, all that can be said is that the local injury to the wall of the vessel which leads to the precipitation of lipoids, together with the presence of the lipoids themselves, results in a proliferation of fibrous connective tissue cells in the intima. With the increase in thickness of the intima and with the appearance of necrosis in its deeper layers, the whole process becomes so complicated that it is impossible to analyze with any chance of accuracy the rôle of individual factors.

In accordance with the discussion presented in the foregoing pages, the pathogenesis of experimental cholesterol arteriosclerosis in rabbits may be summarized as follows: Cholesterol feeding produces general alterations in the blood and secondarily in the nutritive fluid which permeates the walls of the arteries. Among these changes, hypercholesteremia and an increased concentration of lipoids in the nutritive fluid are the only ones which have been recognized up to the present time as being of importance in the formation of lipoid deposits in the arterial walls. In addition to these general changes, some unknown factor inherent in the experimental procedure of cholesterol feeding produces an injury to the walls of the arteries. This injury is responsible for the local histologic changes in the walls of the vessels which precede the appearance of lipoid deposits. In the presence of the appropriate general conditions in the blood and nutritive fluid, lipoids are precipitated from the latter in the injured areas of the arterial walls through the influence of the special local conditions which exist in such areas. The existence of these local conditions is essential to the precipitation of lipoids, and consequently lipoid deposits do not develop in the remaining normal parts of the arteries. The subsequent development of the arterial lesions depends on the continued action of the factors which are responsible for their initiation. However, the presence of precipitated lipoids in the arterial walls constitutes another important local factor, which, together with the original local injury, leads to the subsequent cellular proliferation in the intima. Beyond this point, the development of experimental cholesterol arteriosclerosis is not capable of accurate analysis.

IX. ANATOMIC COMPARISON OF EXPERIMENTAL CHOLESTEROL
ARTERIOSCLEROSIS AND HUMAN ARTERIOSCLEROSIS

From the description of the lesions of experimental cholesterol arteriosclerosis in rabbits which has been given in section IV of this paper, it is obvious that there are certain points of resemblance between them and the lesions of arteriosclerosis in man, but it is equally obvious that there are a number of differences between the two. The gross appearance of the individual lesions in the arteries of the experimental animal is not unlike that of certain lesions of human arteriosclerosis and in some respects their distribution is similar. The experimental arterial lesions frequently involve areas about the mouths of branching arteries, in much the same way as in human arteriosclerosis. Anitschkow [12] and others have pointed out that the localization of the early lesions of experimental cholesterol arteriosclerosis in the aorta shows the closest correspondence with the distribution of the minute lipoid flecks found in the aortas of children, but whether or not the latter represent the initial stage of human arteriosclerosis is, of course, another question. On the other hand, the lesions in the rabbit's aorta tend to become most advanced in the arch, while in man it is a common observation that arteriosclerosis usually reaches its greatest severity in the abdominal portion of the aorta. In the rabbit, the pulmonary artery and its branches are affected to a degree proportional to the severity of the lesions in the aorta, while in man it is well known that the pulmonary artery is usually spared even in the presence of advanced arteriosclerosis in the aorta and elsewhere. In experimental cholesterol arteriosclerosis, the renal arteries are affected rather infrequently, but perhaps the most striking divergence from the distribution of arteriosclerosis in man is to be found in the fact that the cerebral and retinal arteries are never involved in experimental cholesterol arteriosclerosis.

Microscopically, no comparison can be drawn between the very early stages of the arterial lesions in the rabbit and in the human being for the reason that in the latter there is still no general agreement as to what may constitute the earliest change. However, the slightly more advanced lesions of experimental cholesterol arteriosclerosis exhibit a number of points of similarity to certain lesions of human arteriosclerosis. There is a localized thickening of the intima in which doubly refractive and other lipoids are present in extraordinary quantities. Large fat-containing macrophages or foam cells are present in abundance, and there is some proliferation of fibrous connective tissue cells. The lipoids are found within cells and densely strewn through the intercellular ground substance, although they are not concentrated along elastic fibrils in the intima as they so frequently are in human arteries. Among the lipoids, cholesterol and its esters seem to be present in greatest abundance as in

human arteriosclerosis.[d] An even closer resemblance to arteriosclerosis
in man is found in the more mature arterial lesions which develop in
rabbits after a long period of cholesterol feeding followed by a longer
period during which cholesterol is withheld from the diet. In such
lesions, lipoids are not so abundant, foam cells are present in much
smaller numbers, and the intimal thickening is predominantly fibrous.
Altogether, then, the lesions in the arteries, so far as the changes in
the intima are concerned, simulate very closely the lesions of human
arteriosclerosis. However, the same statement cannot be made when the
lesions in the media are taken into consideration.

In rabbits, following cholesterol feeding anisotropic lipoid deposits
sometimes appear in the inner layers of the media before there is any
visible change in the overlying intima, or they may appear at a very
early stage in the development of the intimal lesions. Lipoid deposits
in the media have never been described as a feature of the early stages
of the development of arteriosclerosis in man. When lipoid deposits
in the media occur in human arteriosclerosis, they are found beneath
well developed intimal plaques. Moreover, the lipoids appear first
between the muscle fibers and the adjacent elastic laminae, both of
which remain intact for a considerable time afterward. In the rabbit,
on the other hand, the appearance of the anisotropic lipoids in the
media follows destruction and disappearance of muscle and elastic
tissue. So far as the histologic changes in the media are concerned,
therefore, the arterial lesions of experimental cholesterol arteriosclerosis
do not correspond at all closely with those of arteriosclerosis as it occurs
in man. This fact may account for the apparent reluctance of some
investigators to describe specifically the condition of the media in experi-
mental cholesterol arteriosclerosis, and may explain their preference for
noncommittal descriptions of the arterial lesions in which repeated
references are made, not to the intima or media, but to "the inner
layers of the arterial wall."

In addition to the lesions in the arteries, the associated changes in
other tissues which occur during the development of experimental cho-
lesterol arteriosclerosis must be taken into consideration. It has been
mentioned already that in the usual course of cholesterol feeding in the
rabbit there is brought about an accumulation of anisotropic lipoids in
various situations other than the walls of arteries.[e] Lipoids accumulate
in the cells of the suprarenal cortex, in the liver cells and in the reticulo-
endothelial cells of the liver, spleen, lymph nodes and bone marrow.
These lipoid accumulations appear before any changes are to be found

(*d*) 136, 175, 182. See also the chemical analyses of the lipoids contained in
arteriosclerotic lesions of the human aorta reported recently by Meeker and Jobling
(Arch. Path. **18**:252, 1934).

(*e*) 34, 135, 165, 189.

in the arteries, and they persist as long as cholesterol feeding is continued. This phenomenon has no counterpart in arteriosclerosis in man. There are those who claim that there is an unusual abundance of lipoids in the suprarenal cortex in association with human arteriosclerosis, but this is open to doubt and certainly has never been satisfactorily established. On the other hand, it is admitted by all that no abnormal accumulation of doubly refractive lipoids in the cells of the reticulo-endothelial system or in the liver cells occurs in association with human arteriosclerosis. Attempts have been made to minimize this discrepancy on the ground that experiments can be so arranged as to avoid the accumulation of such excessive amounts of lipoids in the reticulo-endothelial cells during the development of lesions in the arteries. This can be accomplished by feeding very small quantities of cholesterol over a period of several years, but even under these conditions there are found relatively slight but definitely abnormal lipoid accumulations in the cells of the reticulo-endothelial system.[f] Lipoids may disappear almost completely from the reticulo-endothelial cells if cholesterol feeding is discontinued after arterial lesions have been produced, and if a long period of time is then allowed to elapse before the experiment is terminated. However, one can feel perfectly certain that the reticulo-endothelial cells were laden with anisotropic lipoids during the period when the arterial lesions were developing, a condition which is not observed as an accompaniment of the development of arteriosclerosis in man.

The lipoid deposits which may appear in the rabbit's cornea as a result of cholesterol feeding have been described by Versé[163] and others as the analog of the arcus senilis in man. However, the arcus senilis does not occur with great regularity in association with human arteriosclerosis; as the name implies, it is generally associated with old age rather than with arteriosclerosis as such. Accumulations of anisotropic lipoids in the interstitial tissue of the kidneys occur in connection with experimental cholesterol arteriosclerosis. These lesions, which have been described in detail by Bailey[20] and Schönheimer,[135] develop in the absence of any changes in the renal arteries which, as has been pointed out, are seldom affected in the experimental disease. It is evident that no similar lesions in the kidneys are associated with arteriosclerosis in man. Deposits of doubly refractive lipoids have been described as occurring in various other tissues of rabbits in which experimental cholesterol arteriosclerosis has been produced. Such deposits have been found in the skin, subcutaneous tissue and tendons and in the mucosa and submucosa of the gallbladder, bile ducts, stomach and intestine.[f] Although careful search, perhaps, has not been made in human cases, similar accumulations of anisotropic lipoids have never been described as a regular accompaniment of arteriosclerosis in man.

(f) 34, 135, 165, 189.

The obvious purpose of drawing a comparison between the anatomic lesions of experimental cholesterol arteriosclerosis and those of human arteriosclerosis is to determine whether or not the experimental disease can be considered from the anatomic point of view as a reproduction of arteriosclerosis as it occurs in human beings. Enough has been said already to show that the arteriosclerosis produced experimentally in rabbits by cholesterol feeding is not anatomically identical with human arteriosclerosis. The similarity between the arterial lesions in the two instances is very striking, and there need be no hesitation in stating that experimental cholesterol arteriosclerosis resembles arteriosclerosis in man more closely than any experimental lesions of arteries which have yet been produced by procedures other than cholesterol feeding. Nevertheless, the differences must be taken into account.

It is contended by some that the explanation of these differences lies in the normal anatomic differences between the rabbit and man, such, for example, as exists in the structure of the intima of the aorta. But who can say how much should be allowed for such differences in anatomic structure? It is conceivable that the differences between the arterial lesions in the two instances may be explained on this basis. However, one can say only that this explanation is possibly correct or perhaps correct in part, but not necessarily so. The accumulations of anisotropic lipoids in various situations other than the arteries in experimental cholesterol arteriosclerosis, which do not occur in connection with human arteriosclerosis, seem inexplicable on the grounds of anatomic differences. Lesions similar to those observed in cholesterol-fed rabbits (e. g., xanthomas of the skin and internal parts and overloading of the reticulo-endothelial system with various lipoids) are encountered on occasion in the field of human pathology; [g] it is clear that such lesions can develop in the human body, but they do not occur in any close or constant association with arteriosclerosis. The close association of extra-arterial lipoid accumulations with arteriosclerosis is a feature peculiar to the experimental disease.

Altogether, it seems that the anatomic picture of experimental cholesterol arteriosclerosis in rabbits shows predominantly a saturation of the whole body with lipoids. These accumulate in excessive quantities in the cells of the liver, suprarenal cortex and reticulo-endothelial system and, in addition, they are precipitated in the intercellular substance of various tissues wherever the local conditions are suitable, whether in the arteries or elsewhere. On the other hand, the whole anatomic picture of human arteriosclerosis exhibits a process in which the rôle of the lipoids is confined almost exclusively to their much less conspicuous accumulation in the arterial lesions. All of this suggests very strongly that the part played by the lipoids in the development of arteriosclerosis,

(g) 70, 165.

whatever that part may be, is greatly exaggerated in experimental cholesterol arteriosclerosis as compared with arteriosclerosis in man. Recognition of this general difference is of essential importance to a correct interpretation of the significance of the experimental data.

The only definite conclusion which can be drawn from an anatomic comparison of experimental cholesterol arteriosclerosis with human arteriosclerosis is that the two are not completely identical. An exact evaluation of the importance of the differences will not be attempted; on this point there can be no final judgment at present. Certainly one is not justified in taking such an insecure position as that of Anitschkow, who sweeps aside all differences with the mere statement that "none of these differences is of an essential nature."[12] One can proceed further only on the ground that the anatomic similarity between experimental cholesterol arteriosclerosis and human arteriosclerosis should permit one to expect some features of similarity in their etiology and pathogenesis. Nevertheless, the anatomic differences between the two should be sufficient caution against the hasty conclusion that their etiology and pathogenesis are of necessity exactly or even nearly identical, or that the etiologic factors which may be common to the experimental and human diseases must operate with equal intensity in both instances.

X. SIGNIFICANCE OF EXPERIMENTAL DATA IN RELATION TO ETIOLOGY AND PATHOGENESIS OF HUMAN ARTERIOSCLEROSIS

Various factors of possible importance in the etiology of experimental cholesterol arteriosclerosis have been considered individually in section VI of this paper, and the pathogenesis of experimental cholesterol arteriosclerosis in rabbits has been discussed in section VIII. On the basis of the available experimental evidence brought forward in those discussions, three main factors were implicated in the production of the lesions in the arteries of rabbits, namely, the presence of considerable quantities of cholesterol in the diet, the existence of hypercholesteremia and the occurrence of injury to the arteries. To assume that these same three factors are all in operation in the development of human arteriosclerosis, or that they operate in man in exactly the same way as in the rabbit, is entirely without justification, not only for the reasons pointed out in the foregoing section (section IX) but also because there are certain differences between the rabbit and man which have not yet been touched on and which place further obstacles in the path of any attempt to make a direct application of the inferences drawn from the experimental data.

Some differences between the rabbit and man which are of obvious importance in this connection may be pointed out briefly. Among these is the fact that the rabbit is a herbivorous animal while man is omnivorous. Cholesterol is completely lacking in vegetable diets, although

phytosterol, the plant relative of cholesterol, is present in comparatively small amounts. On the other hand, cholesterol is present in varying quantities in the normal diets of omnivorous animals. Perhaps related to this difference in normal diet is the extreme tardiness with which the rabbit excretes exogenous cholesterol as compared with the rapid excretion of cholesterol in man.[h] This deficiency of the rabbit's excretory apparatus serves to explain the ease with which hypercholesteremia and hyperlipemia can be produced in rabbits by means of cholesterol feeding. [In man, on the contrary, large quantities of cholesterol in the diet have only a relatively slight and temporary effect on the level of cholesterol in the blood,[i] owing apparently to the facility with which cholesterol is disposed of by the human mechanism. Another difference of importance is that which exists between the normal cholesterol content of the blood of the rabbit and that of man. As may be seen from the data collected by Weidman and Sunderman,[170] the normal range of the blood cholesterol in rabbits, as determined by various investigators, is far below that found in man by corresponding methods of cholesterol estimation. Taking the median point of the normal range in each case, the cholesterol content of human blood is approximately twice as great as that of the rabbit's blood. The last difference which need be mentioned is of no less importance than the others. The typical spontaneous arteriosclerosis of rabbits shows a lack of any lipoid deposits in the arterial lesions. Using the word "spontaneous" in the same sense, the typical spontaneous arteriosclerosis of man is characterized almost constantly by the presence of more or less extensive accumulations of lipoids in the affected parts of the arteries. One can hardly avoid the suspicion that this divergence between the respective types of spontaneous arteriosclerosis may be related to the difference between the normal lipoid content of the blood of the rabbit and that of the human being.

These differences between the rabbit and man complicate the interpretation of the experimental results and render it impossible to evaluate the significance of the experimental data without making careful allowances for the differences which have been mentioned. Such being the case, it is rather surprising that any one should consider it fortunate that the rabbit is the animal in which the cholesterol feeding experiments have proved successful. Nevertheless, Anitschkow [12] has contended that the rabbit is an especially suitable animal for experimental investigations on arteriosclerosis simply because it is not subject to the spontaneous development of arterial lesions resembling those of human arteriosclerosis. Actually, this is one of the best reasons for subjecting to the closest possible scrutiny the inferences drawn from the experimental data obtained in rabbits.

(*h*) 28, 135.
(*i*) 28, 52, 107, 144.

In order to produce arterial lesions resembling those of human arteriosclerosis in an animal in which the spontaneous arteriosclerosis of "old age" is of a quite different type, one must really do two distinct things: First of all, the animal must be converted into one which is susceptible to the development of arterial lesions of a character quite foreign to it. In this artificially prepared animal, a second factor or group of factors must then be introduced for the actual production of the lesions in the arteries. Obviously, the second step is the only one that could be of any importance in relation to human arteriosclerosis. But it happens that all of this can be accomplished in the rabbit by the seemingly simple procedure of feeding large quantities of cholesterol. Now, under these conditions, it is difficult to determine from the experimental data alone what it is that converts the rabbit into a susceptible animal and what it is that is responsible for the actual production of the characteristic lesions in the arteries. On the basis of the evidence brought forward in this paper, it seems most likely that the rabbit is rendered susceptible to the development of arterial lesions simulating those of human arteriosclerosis by the elevation of its blood cholesterol level, which is normally much lower than that in man and which is raised by cholesterol feeding. The actual cause of the lesions in the arteries is an injury to the arterial walls which is produced by feeding large quantities of cholesterol or cholesterol-rich foods, but which is not caused directly by the resulting hypercholesteremia. If all of this is true, as is probably the case, it follows that the hypercholesteremia as the preparatory factor has no corresponding importance in relation to human arteriosclerosis, but the injury as the exciting factor must be of the greatest significance. As I shall show presently, the available evidence derived from the study of human material corresponds precisely with this idea.

The major difficulties in estimating the significance of experimental arteriosclerosis produced in animals would be removed by selecting for experimentation an animal species which is closely related to man and which is naturally subject to the development of a type of spontaneous "old age" arteriosclerosis which resembles human arteriosclerosis as closely as possible. The obvious aim, then, would be to produce prematurely in such an animal lesions of the arteries of a character corresponding with its own type of spontaneous arteriosclerosis. Of course, the possible spontaneity of the lesions supposedly produced by the experimental procedure would have to be excluded by the examination of adequate numbers of control animals. So far as I am aware, successful experiments of this nature have never been reported. However, if positive experimental results could be obtained under the conditions described, the significance of those results would not remain long in doubt.

Returning to the cholesterol feeding experiments in rabbits, I wish to reemphasize the fact that the differences between the experimental animal and man, added to the anatomic differences between experimental cholesterol arteriosclerosis and human arteriosclerosis, render the problem of interpretation far from simple. In the past, the difficulties of interpretation have never been given adequate consideration, and inferences regarding human arteriosclerosis have been drawn with the greatest abandon from the experimental data. A much too enthusiastic and zealous faith in the validity of the experimental results has led to the blind acceptance of conclusions which have been quite misleading. The outstanding example of this free exercise of faith is the general belief that hypercholesteremia is a factor of paramount importance in the etiology of arteriosclerosis in man, a belief which is based entirely on the probability that hypercholesteremia is essential to the development of experimental cholesterol arteriosclerosis in rabbits.

Anitschkow [j] has adroitly turned to advantage the difficulties of interpretation which he is willing to recognize as such. He has expressed the belief that the unique experimental procedure, the relatively short duration of the experiments and the differences between the rabbit and man are just sufficient to explain the differences between the total picture of experimental cholesterol arteriosclerosis and that of human arteriosclerosis. Having offset all of these differences, one against another, he has proceeded with the apparent conviction that the etiology and pathogenesis of experimental cholesterol arteriosclerosis and of human arteriosclerosis are essentially the same. Such an assumption is not justified by the known facts. Aschoff,[17] on the other hand, pointing especially to the difference in cholesterol excretion between the rabbit and man, has made the following comment. "For this reason one should not attempt to apply the conclusions of experiments with rabbits too freely, and their interpretation in human beings must be accepted with reserve." This attitude of caution seems imperative if gross misinterpretations are to be avoided.

When all of the circumstances are taken into consideration, it becomes perfectly obvious that the application of conclusions drawn from the experiments cannot be justified without some evidence of the existence of comparable conditions in the human being. A proper appreciation of the significance of the experimental results can be gained only through correlation of the experimental data with corresponding data derived from the study of human material. Accordingly, it will be necessary to consider individually the principal factors involved in the development of experimental cholesterol arteriosclerosis and to

(j) 8, 12.

determine whether or not there is any indication of the operation of similar factors in the development of human arteriosclerosis.

(*a*) *Cholesterol in the Diet.*—In section VI of this paper, reasons have been given for the conclusion that the presence of considerable quantities of cholesterol in the rabbit's diet is an essential factor in the production of experimental cholesterol arteriosclerosis. In the same section it was shown that one important result of the administration of cholesterol in the diet is hypercholesteremia, a condition which was assumed to be also an essential factor in the development of the arterial lesions in rabbits. Is there now any reason for believing that cholesterol in the diet of human beings plays a comparable rôle in the development of human arteriosclerosis? This question can be answered at once in the negative on the following grounds:

In the human being, in contrast with the rabbit, cholesterol is a normal constituent of the diet, being contained in considerable quantities in eggs, milk, butter and meat. The amount of cholesterol ingested undoubtedly varies considerably between persons, and from time to time in any single person, but these variations in the cholesterol content of the diet have never been shown to have any significant effect on the cholesterol content of the blood. Furthermore, the deliberate addition of cholesterol to the food of normal subjects does not produce a sustained hypercholesteremia; the effect on the level of cholesterol in the blood is relatively slight and transitory.[k]

Now, in spite of the differences in the cholesterol content of the diets of different persons and of the various diets of different races, it has never been shown that those persons or races which subsist on diets poor in cholesterol are by any means protected against the development of arteriosclerosis. Nor has it been shown that those persons or races which develop arteriosclerosis at an earlier age or in more severe degree than others are especially partial to foods which are rich in cholesterol. On this point one may quote some remarks of Weiss and Minot,[174] who have reviewed the literature bearing on nutrition in relation to arteriosclerosis. "It cannot be stated that overnutrition with lipoid and fatty substances plays a rôle in the production of arteriosclerosis in normal man. Statements that fat is responsible for arteriosclerosis are not lacking in the literature, but these statements are not based on controlled observations, and they seem to be strongly influenced by the imbibition theory of arteriosclerosis proposed by Virchow and amplified recently by Aschoff and Anitschkow. Caution is necessary at this point lest we fall into the so often dangerously applied *post hoc propter hoc* method of reasoning." As Weiss and Minot have pointed out, all of the evidence which has been brought forward in the attempt to demonstrate a relationship between

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(*k*) 28, 52, 107, 144.

the cholesterol content of the diet and the development of arteriosclerosis in man is equivocal, and therefore, far from convincing. Indeed, it would hardly influence an opinion not already prejudiced in favor of the idea.[1]

In considering the significance of the experimental data, the results of cholesterol feeding experiments in animals other than the rabbit cannot be overlooked. It is especially important to bear in mind that animals such as cats and dogs which, like man, fail to show marked hypercholesteremia following cholesterol feeding, do not show any changes in their arteries after prolonged periods of feeding on diets rich in cholesterol. The uniform failure of all attempts to produce arterial lesions by this method in cats, dogs, foxes and monkeys speaks strongly against the idea that cholesterol in the diet is a factor of any importance in the etiology of human arteriosclerosis. The negative results obtained in these animals seem more properly applicable to the human being than do the positive results obtained in rabbits.

The considerations set forth in the preceding paragraphs lead to the conclusion that the results of cholesterol feeding experiments in rabbits do not constitute a valid reason for believing that an excess of cholesterol in the diet plays a rôle in the etiology of arteriosclerosis in man. No convincing evidence in support of such a belief exists at present.

(b) *Hypercholesteremia.*—In section VI (b) of this paper it was pointed out that a well marked or even extreme hypercholesteremia, and indeed a general hyperlipemia, almost constantly precedes and accompanies the development of experimental cholesterol arteriosclerosis. In the same place, the tentative conclusion was reached that some elevation of the blood cholesterol is necessary to the development of the fatty arterial lesions in rabbits. The probability that this is true is supported by certain theoretical considerations brought forward in section VIII, where it was also concluded that hypercholesteremia is effective only in the presence of local injury to the walls of the arteries. These conclusions, of course, applied only to the development of experimental cholesterol arteriosclerosis in rabbits. In man, on the contrary, no analogous changes in the blood are found to occur in connection with arteriosclerosis. No change in the blood lipoids has been shown to exist as a sign of the impending development of arteriosclerosis in man, nor has any change in the lipoid content of the blood been found as a constant accompaniment of any stage in the progress of the disease.

It is sometimes stated that hypercholesteremia is regularly associated with arteriosclerosis in man, but such statements are based on a litera-

(1) The status of this question is not altered by the recent publication of Rosenthal (Arch. Path. **18**:473, 1934). The evidence which he has advanced is of the same character as that adduced in the previous literature and is open to the same objections.

ture much of which has no direct bearing on the question. Some publications which are frequently referred to in this connection have nothing to do with arteriosclerosis as such, but are concerned with various other diseases in which hypercholesteremia is known to occur and with which arteriosclerosis may be associated. Other reports deal with the occurrence of hypercholesteremia in association with high blood pressure. In some of these publications, cases of "essential" hypertension have been selected for study, but in others the clinical cases have been chosen with high blood pressure as the only qualification, so that diseases of the greatest variety have been included (even diabetes mellitus!). These studies of hypertension, whether in carefully selected cases or otherwise, have yielded the most conflicting results. Some investigators have found hypercholesteremia in a proportion of their cases varying from 13 to 76 per cent,[m] while others have found normal blood cholesterol levels in practically all cases of their series.[n] Some of these authors believe that hypercholesteremia causes hypertension; some believe that hypertension causes hypercholesteremia; others contend that the two are not related. Whatever may be the truth of the matter, it is obvious that these data prove absolutely nothing regarding the occurrence of hypercholesteremia in association with anatomic lesions of the arteries.

The publications which contain direct information as to the occurrence of hypercholesteremia in association with human arteriosclerosis are relatively few. Weltmann,[176] without giving data on individual patients, stated that in general he had observed increased blood cholesterol in his arteriosclerotic patients, some of whom had apparently shown hypertension. Koulkov and his collaborators [77] made determinations of the blood cholesterol in 50 arteriosclerotic patients and found values above 160 mg. per hundred cubic centimeters in 20 cases. Mjassnikow [102] observed elevation of the blood cholesterol level in 30 cases of arteriosclerosis among the 70 which he studied. He found hypercholesteremia most consistently in patients suffering from far advanced arteriosclerosis of the aorta and coronary arteries, while it occurred with rapidly diminishing frequency in the less severe cases. On the other hand, Stepp [150] could find no abnormality in the levels of cholesterol in the blood in cases of arteriosclerosis without hypertension. In the cases of arteriosclerosis studied by Denis,[39] the cholesterol content of the blood was normal regardless of the presence or absence of hypertension.

From all of this, one can conclude only that hypercholesteremia may be found in certain cases of advanced arteriosclerosis and not in others. All of these cases are properly described as "advanced," for

(*m*) 3, 54, 122, 168, 173, 181.
(*n*) 58, 91, 102.

in all instances the diagnosis of arteriosclerosis was made from clinical study alone, and it is obvious that arteriosclerosis of sufficient degree to be recognized clinically is far past the initial stages. Now, if, as Mjassnikow [102] claimed, the incidence of hypercholesteremia is highest among those patients with the most extreme arteriosclerosis and lowest among those least affected, the occurrence of hypercholesteremia might more logically be regarded as the effect rather than the cause of arteriosclerotic changes which have already developed. Bruger and Poindexter,[27] in a recent study of the plasma cholesterol in relation to the development of arteriosclerosis in obese persons, reached practically the same conclusion. However, one need not dwell on this point, for the available data, including the results of those who seem most eager to demonstrate an elevation of the lipoids in the blood, fail to give convincing evidence that hypercholesteremia occurs in anything approaching a close association with arteriosclerosis. On this question, then, a final conclusion may be expressed in agreement with such disinterested authorities as Bürger,[28] Gardner [51] and Peters and Van Slyke,[119] who are satisfied to dismiss the subject with the simple but significant statement that hypercholesteremia is not found with any regularity in association with arteriosclerosis in man.

The fact that an elevation of the cholesterol or of other lipoids in the blood is not found as an accompaniment of arteriosclerosis in man constitutes a strong argument against the idea that such a disturbance of the blood lipoids is the cause of arteriosclerosis or that it is an essential factor in the development of the arterial lesions. Indeed, if hypercholesteremia were the cause of arteriosclerosis or an indispensable factor in the etiology of the disease, it would necessarily precede the development of lesions in the arteries, but this has never been shown to occur, nor is it even suggested by any recorded observations. Actually, the only reason for suspecting hypercholesteremia as a factor in the etiology of human arteriosclerosis lies in the results of the cholesterol feeding experiments in rabbits. While these results form adequate grounds for the suspicion, they do not and cannot form adequate grounds for the conclusion that hypercholesteremia is necessary to the development of arteriosclerosis in man. In view of the data derived from the study of human material, it seems highly probable that arteriosclerosis in man can and usually does develop without deviation of the cholesterol content of the blood beyond the normal limits of variation. In any event, there is no valid evidence to support any other conclusion.

The apparent discrepancy between the two conclusions, that hypercholesteremia is an indispensable factor in the etiology of experimental cholesterol arteriosclerosis in the rabbit, and that it is not necessary to the development of arteriosclerosis in man, is not by any means inexpli-

cable. As I have pointed out, the level of cholesterol in the blood is normally very low in rabbits; in human beings, on the other hand, it is normally much higher. Not only is cholesterol present in greater concentration in human plasma, but it normally stands at a level not far from the point of saturation.[175] Under these conditions, it is not surprising that a blood cholesterol content within the normal limits should suffice for the precipitation of cholesterol in the walls of human arteries wherever the local conditions are favorable for its accumulation. In the rabbit, following cholesterol feeding, the content of cholesterol in the blood is increased greatly, often to an extreme degree. This corresponds with another observation to which I have called attention, that the anatomic lesions of experimental cholesterol arteriosclerosis give clear indication of an abnormal saturation of the whole body with lipoids. The abundance and widespread distribution of the lipoid deposits are out of all proportion to anything of a similar nature that occurs in association with human arteriosclerosis.

These facts provide a reasonable explanation for the phenomena actually observed in the rabbit, and in man. Thus, in the rabbit, in which the normal level of cholesterol in the blood is low, there occurs a type of spontaneous arteriosclerosis the lesions of which are practically devoid of stainable fat. In the cholesterol feeding experiments when the blood lipoids are elevated, cholesterol and other lipoids are deposited in abundance, not only in the lesions in the arteries but also in various other tissues throughout the body. In man, on the contrary, the normal level of cholesterol in the blood is relatively high, so that the lesions which develop in the arteries may become impregnated with lipoids without any change in the concentration of cholesterol in the blood. At the same time, since the blood cholesterol is not elevated, there is no accumulation of lipoids in the cells of the reticulo-endothelial system nor any abnormal lipoid deposits in other tissues. Of course, it is implied that lipoids are deposited in the walls of human arteries, as in the arteries of rabbits, only in those areas in which the local conditions have been rendered suitable for the precipitation of lipoids by the prior occurrence of local alterations in the walls of the vessels, a proposition which I shall presently defend. This tentative scheme of the relationship between experimental cholesterol arteriosclerosis and human arteriosclerosis brings into accord what might otherwise appear to be conflicting conceptions. It is of greater importance that this end is accomplished without doing violence to the facts as they are known at present.

In discussing the pathogenesis of experimental cholesterol arteriosclerosis in rabbits, it has been concluded that hypercholesteremia acting alone does not produce the experimental lesions of the arteries. The lesions develop only when hypercholesteremia and local injuries to the

walls of the arteries are combined. The experiments therefore give
no reason for believing that hypercholesteremia when it occurs in human
beings can of itself produce arteriosclerosis. However, it might be
supposed on the basis of the experimental evidence that hypercho-
lesteremia in man would accelerate the development of whatever arterio-
sclerotic changes happened to be already in progress. This supposition
seems reasonable enough, and yet actual observation of patients in whom
hypercholesteremia is known to exist does not offer much support for
the idea. At the same time, a study of such patients provides con-
vincing evidence to show that hypercholesteremia of itself is not a cause
of arteriosclerosis.

I am aware that the high incidence of arteriosclerosis among patients
with diabetes mellitus is frequently cited as evidence of the ability of
hypercholesteremia to produce arteriosclerosis in man. This is a disease
in which hyperlipemia and hypercholesteremia are well known to occur,
but it is hardly necessary to point out that the metabolic disturbance
in diabetes is not by any means confined to a disturbance of lipoid
metabolism or to an elevation of the lipoids in the blood. The well
recognized tendency of diabetic patients to have arteriosclerosis is not
necessarily due to the existence of hypercholesteremia. Hyperglycemia,
ketosis or the frequent occurrence of infections in association with dia-
betes could just as well be held responsible. Moreover, it is not at all
clear that the severity of the arteriosclerotic changes runs parallel with
the degree of elevation of the blood lipoids. With this point in mind,
Hunt [63] followed the progress of a group of diabetic patients and found
that she could not establish any direct relationship between the elevation
of the blood cholesterol level and the degree of arteriosclerosis which
developed. Indeed, in her series of cases, the most advanced arterio-
sclerotic changes appeared in those patients whose average blood cho-
lesterol level had been lowest.

If hypercholesteremia in man really can produce arteriosclerosis or
can exert an appreciable influence on its development, abundant oppor-
tunities to observe these effects are offered by the occurrence of hyper-
cholesteremia in association with a variety of conditions other than
diabetes mellitus, e. g., pregnancy, hypothyroidism, obstructive jaundice,
certain types of nephritis and lipoid nephrosis.[o] In spite of these oppor-
tunities, it has never been shown that the degree of arteriosclerosis
found at autopsy in patients with such conditions is appreciably greater
than might be expected in any other subjects of the same age; certainly
to ordinary observation no difference is apparent. This is especially
striking in children who come to autopsy after suffering even for a
period of several years from lipoid nephrosis with constant or inter-
mittent hypercholesteremia of extreme degree. In these cases, there are

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(o) 28, 119.

no apparent alterations in the arteries, or none which might not be found in children of the same age dying from any other disease.[25] These negative observations in patients with outspoken hypercholesteremia of considerable duration constitute the strongest sort of evidence against the idea that hypercholesteremia acting alone can cause arteriosclerosis. There is every reason, then, to conclude, in agreement with Bürger,[28] that hypercholesteremia of itself cannot be regarded as a cause of arteriosclerosis in man. Although hypercholesteremia, when it occurs, might be expected on theoretical grounds to accelerate the development of arteriosclerotic changes which have already been initiated, there is little evidence at present to indicate such an effect.

Now, in spite of the fact that the data derived from the study of human material offer no support whatever, Anitschkow [p] has insisted that hypercholesteremia resulting from some disturbance of cholesterol metabolism is responsible for the development of arteriosclerosis in man, an idea based entirely on the results of cholesterol feeding experiments in rabbits. He has always avoided stating directly that hypercholesteremia acting alone can cause arteriosclerosis in human beings and has admitted that factors which produce local injury to the arterial walls must be of importance, but only of secondary importance. He has expressed the belief that hypercholesteremia of very slight degree may be sufficient to cause arteriosclerosis "provided only that it is of long duration and associated with other injurious factors." [12] One cannot deny the possibility that fluctuations of the blood cholesterol so slight as to escape detection may occur. But the chemical methods in general use are accurate enough to determine the normal fluctuations of the level of the cholesterol in the blood. It is probable, therefore, that a significant hypercholesteremia could also be detected, but it has not been found. Moreover, as I have shown, it is unnecessary to assume that even a slight degree of hypercholesteremia is essential to the development of human arteriosclerosis.

Anitschkow's further suggestion of a "primary" disturbance of cholesterol metabolism as the underlying cause of arteriosclerosis in man is, of course, merely an invention to provide some reason for the hypothetical elevation of cholesterol in the blood. This idea is not supported even by the experimental evidence, much less by observations on human beings. Nevertheless Anitschkow,[12] in speaking of the etiology of human arteriosclerosis, has made the following statement: "On the basis of all these experimental results, it may therefore be regarded as definitely established that cholesterin, or rather a disturbance of the cholesterin metabolism, is of decisive significance as far as the genesis of atherosclerosis is concerned." For the reasons that have been given, one cannot accept the experimental evidence alone as proof of this point. If a disorder of cholesterol metabolism is to have any effect on the

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(*p*) 8, 12.

arteries, it must find some expression in changes in the blood, but evidence of the existence of such changes in association with the development of arteriosclerosis in man is entirely lacking. Indeed, belief in the participation of a disturbance of lipoid metabolism in the etiology of human arteriosclerosis has always rested on little more than opinion, and the nature of the supposed metabolic disturbance has never been clearly defined. No one considers it necessary to assume a disorder of calcium metabolism to account for the deposition of calcium in the walls of arteries [175] even when this occurs in areas where there is no very obvious evidence of previous injury (e. g., Mönckeberg's sclerosis), but there seems to be a general impression that a disturbance of lipoid metabolism must be postulated to explain the accumulation of lipoids in the lesions of arteriosclerosis. This impression may prove to be correct, but there is no concrete evidence at present to show that it is. In any event, the cholesterol feeding experiments provide no valid reason for believing that a disturbance of cholesterol or lipoid metabolism plays any part in the etiology of human arteriosclerosis. Moreover, there is no definite or concrete evidence from observations on human beings to support the idea. If a disturbance of lipoid metabolism participates in the etiology of arteriosclerosis in man, the fact remains to be demonstrated in the future.[p1]

The conclusions which have been drawn in the preceding paragraphs should by no means be interpreted as a deterrent to the further search for alterations in the blood to which importance could be attached in connection with the etiology of human arteriosclerosis. In the past, attention has been concentrated on possible quantitative changes in the lipoid constituents of the blood plasma, but it may be that qualitative changes are more important. It is possible that alterations may occur in the physicochemical state of the lipoids, perhaps in their dispersion or in their relation to other colloids in the plasma. An interesting lead in this direction is furnished by the work of Alvarez and Neuschlosz,[3] which suggests the possibility that the degree of saturation of the plasma with cholesterol may be of importance. Judging from their figures, one would conclude that the degree of saturation or of supersaturation is independent of the absolute quantity of cholesterol in the blood, and if this is the case, it would seem that the whole colloidal system of the blood plasma, complex as it is, would have to be brought into consideration.

(p1) In his recent publications, Rosenthal (Arch. Path. **18**:473 and 660, 1934) has discussed at great length the relationship of cholesterol metabolism to the development of arteriosclerosis. Like many previous authors, he has taken it practically as a foregone conclusion that disturbances of cholesterol metabolism constitute a factor of first importance in the etiology of the disease. However, he has not advanced any conclusive evidence whatever to support this contention, as careful examination of his data and of the references to the literature will show.

This is merely a suggestion. Other possibilities may be imagined, but the final solution of this problem must await the results of further investigation.

Before the subject of hypercholesteremia is dismissed, brief comment may be made on the results of the experiments in which interference with the reticulo-endothelial system or with the endocrine organs has been combined with cholesterol feeding. These results have been interpreted in certain quarters as indicating that the reticulo-endothelial system and the endocrine system are intimately concerned in the etiology of human arteriosclerosis. These conclusions do not necessarily follow. Again one is confronted with the necessity of making careful comparisons between the conditions associated with the development of experimental cholesterol arteriosclerosis and those with the development of human arteriosclerosis before conclusions are drawn. In the cholesterol feeding experiments in rabbits, the reticulo-endothelial cells become loaded with lipoids before any lesions make their appearance in the arteries, while in man no similar phenomenon is observed in connection with the development of arteriosclerosis. Now if the functional capacity of the reticulo-endothelial system in the rabbit is deliberately reduced, the development of experimental cholesterol arteriosclerosis may well be accelerated, but this constitutes no good reason for assuming that the same is true of human arteriosclerosis in which the reticulo-endothelial cells are not observed to participate in any way, and in which the etiologic conditions, especially as regards the rôle of the lipoids, are not capable of such close comparison. The assumption is rendered even more precarious by the lack of any confirmatory evidence from the study of human material.

Among the effects on the development of experimental cholesterol arteriosclerosis which have been attributed to the influence of various endocrine organs, those which follow interference with thyroid function are the best established and apparently the most definite. Hyperthyroidism and hypothyroidism, experimentally induced, tend respectively to retard and to accelerate the development of experimental cholesterol arteriosclerosis. As has been pointed out, the protective effect of the experimental administration of thyroid gland preparations is apparently related to an increased ability thus conferred on the rabbit to dispose of the excessive amounts of exogenous cholesterol introduced into the diet. The resulting hypercholesteremia is not nearly so extreme as in control animals fed on the same diet. To infer from this information that thyroid function plays a part in the etiology of human arteriosclerosis is unjustified, since the etiologic significance of hypercholesteremia in human arteriosclerosis is extremely doubtful, to say the least, and certainly is not comparable with that of hypercholesteremia in the experimental disease. Moreover, even though hyper-

thyroidism and hypothyroidism in man have been recognized for a long
time, and although it is now known that each has a characteristic effect
on the level of cholesterol in the blood,[119] it has never been shown or
even suggested that the development of arteriosclerosis in man is affected
either favorably or unfavorably by these disturbances of thyroid func-
tion.   In view of these facts, the experimental data cannot be regarded
as valid evidence of the participation of the thyroid gland in the etiology
of human arteriosclerosis.   The experimental results obtained through
interference with other organs of internal secretion are of equally
doubtful significance.

The effect of iodides is similar to that of preparations derived from
the thyroid gland.   The administration of iodides reduces the intensity
of the hypercholesteremia which follows cholesterol feeding in rabbits,
and it retards the development of lesions in the arteries.   It seems clear
that the iodides exert their influence through an effect on thyroid
activity, for their administration is without result in thyroidectomized
animals.   Thus, once again, the production of the experimental result
is chiefly dependent on the effect of thyroid activity in facilitating the
disposal of exogenous cholesterol.   Therefore, the arguments advanced
in the preceding paragraph apply here, too, with equal force and render
it impossible to predict with confidence on the basis of the experimental
findings that the administration of iodides would be of any value in the
prevention or treatment of arteriosclerosis in man.   Put to the test in
the treatment of human arteriosclerosis through the course of many
years, iodides have not proved of definite therapeutic value.   "It is very
probable, as pointed out by Cushny, that the good effects of iodine when
observed at all were noticed in cases in which arteriosclerosis was
associated with syphilis" (Wyckoff [186]).

(c) *Injury to the Arteries.*—In section VI *f* and in section VIII of
this paper, reasons have been given for the conclusion that the occur-
rence of injury to the walls of the arteries is an essential factor in the
development of experimental cholesterol arteriosclerosis in rabbits.   It
has also been shown that the occurrence of local injury is the primary
event, which is followed subsequently by the precipitation of lipoids in
the injured area and by cellular proliferation in the intima.   Almost
all of the considerations on which these conclusions were based apply
quite as appropriately in human arteriosclerosis.   There is every reason
to believe that local injury to the walls of the arteries is an essential
factor and the primary event in the development of arteriosclerosis in
man.   Not only is this suggested by the experimental results, but all
pertinent facts concerning human arteriosclerosis are consistent with
this conclusion.   It will hardly be necessary to repeat in full detail the
arguments which have been set forth in discussion of this point in con-
nection with the etiology of experimental cholesterol arteriosclerosis.

However, I may recapitulate briefly, applying the same lines of reasoning to the development of arteriosclerosis in man, and commencing as before with a consideration of the process by which lipoids accumulate in the walls of the arteries.

The lipoids which appear in the walls of the arteries in human arteriosclerosis accumulate in such large quantities that they can hardly be considered as the products of the breakdown of tissue at the sites of the lesions. Of course, a small part of the lipoid material may arise in this way, but it is clear from chemical analyses of human arteries that the bulk of the lipoids must come from elsewhere [q] and therefore must be brought by way of the blood stream. It is evident that the lipoids contained in the blood plasma enter the arterial wall with the nutritive tissue fluid which is derived from the plasma. This fluid must be the immediate source of the lipoids which accumulate in the lesions of arteriosclerosis.

Since the function of the nutritive fluid is to nourish the tissue of the walls of the vessels, it is obvious that it must permeate freely through all parts of the walls. It seems highly probable that this fluid normally carries with it the various lipoids which are contained in the blood plasma. Nevertheless, lipoid deposits do not occur in the walls of normal arteries, and as has been pointed out, they do not occur in normal human arteries even when there is prolonged hypercholesteremia which presumably increases the lipoid content of the nutritive fluid. In arteriosclerotic vessels, lipoid accumulations are not formed everywhere diffusely but only in localized and restricted areas. The latter fact could not be explained by an elevation of the lipoid content of the blood plasma and of the nutritive fluid even if hyperlipemia could be shown to occur in regular association with arteriosclerosis in man. Whether or not there occurs any change in the lipoids of the blood in human arteriosclerosis, it is evident that the local conditions which exist in the wall of an artery at the sites where lipoids are deposited are different from those which obtain in the normal parts of the same artery where no lipoid deposits occur. The local conditions which exist in the arterial walls at the sites of lipoid deposition and which do not exist elsewhere must constitute the immediate cause of the actual precipitation of lipoids from the colloidal state in which they enter the walls of the vessels with the nutritive fluid. It is equally obvious that the appropriate local conditions must exist before any precipitation of lipoids takes place.

The possibility that the appropriate local conditions necessary for the precipitation of lipoids exist normally in certain parts of normal arteries has been mentioned in the discussion of the pathogenesis of experimental

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(*q*) 136, 175, 182.

cholesterol arteriosclerosis in rabbits. This suggestion was dismissed since it was inconsistent with the known facts and was supported only by the results of experiments which evidently had been misinterpreted. The idea is equally inconsistent with the facts concerning human arteriosclerosis so that it can be dismissed here, too, without further discussion. The only alternative and really the only reasonable conclusion is that the local conditions in the walls of the arteries which are responsible for the precipitation of lipoids develop as the result of changes in the walls of the arteries themselves. These local alterations which must occur before the deposition of lipoids becomes possible constitute the initial stage of the development of arteriosclerosis in man.

Just as in the case of the rabbit, the following general statement can be made: The occurrence of localized lipoid deposits in the walls of the arteries is dependent on the coexistence of two sets of conditions. One set includes the conditions which must obtain in the blood and secondarily in the nutritive fluid which permeates the walls of the vessels. These are the general conditions. The second set comprises those conditions which must exist locally in the walls of the arteries at the sites where lipoid deposits are to occur. These are the local conditions. When both the local and the general conditions are suitable, lipoid deposits develop.

In the case of the human being, in contrast with the rabbit, it seems probable that the general conditions are satisfied in the normal person. There is no evidence to show, nor any reason to believe, that an elevation of the blood lipoids is necessary to the development of human arteriosclerosis, and no other changes in the blood have ever been demonstrated in constant association with arteriosclerosis in man. One is justified, therefore, in drawing the tentative conclusion that the general conditions necessary for the formation of lipoid deposits in the walls of the arteries are normally existent in the normal human being. On the other hand, corresponding with the state of affairs in the rabbit, the necessary local conditions almost certainly do not exist normally in man. As I have pointed out, the occurrence of preliminary local alterations in the walls of human arteries prior to the formation of lipoid deposits can hardly be doubted, and indeed may be regarded as an established fact. This is admitted by almost every one, although it is seldom emphasized. Thus, with respect to the primary local changes in the arterial walls, experimental cholesterol arteriosclerosis in the rabbit is quite capable of comparison with arteriosclerosis in man. The important difference lies in the fact that in the human being one is forced at present to regard the occurrence of the appropriate local alterations in the walls of the arteries not only as the initial stage of the development of arteriosclerosis, but as the one abnormal factor responsible for the subsequent train of events.

To determine the cause and nature of these initial local changes in the arterial walls is obviously a problem of paramount importance in the investigation of human arteriosclerosis. So far as these changes are concerned, comparable conditions seem to obtain both in the development of experimental cholesterol arteriosclerosis and in that of human arteriosclerosis, so that the experimental data may properly be drawn on for assistance in the elucidation of this problem. The experimental studies on cholesterol-fed rabbits have been misleading in some respects, for reasons which I have attempted to make clear. This fact, however, constitutes no argument for rejecting the conclusions which can be applied with justification. Therefore, one can accept as valid the experimental evidence which bears on the important question under consideration. As I have shown, the experiments yield evidence which suggests very strongly that some sort of injury is responsible for the preliminary local changes in the walls of human arteries which are essential to the subsequent formation of lipoid deposits. Many of the experimental measures which have been employed for the production of injury to the arteries of rabbits seem to have no direct significance as applied to man, but this does not diminish the weight of the evidence in support of the general conclusion that the primary local alterations in the arterial walls which lead to the subsequent development of the lesions of arteriosclerosis are the result of some sort of injury to the arteries, an injury to which in man the intimal layer seems to be especially susceptible.

That this inference is consistent with the knowledge of arteriosclerosis as it occurs in man is attested by the fact that many investigators have reached essentially the same conclusion from the study of human material alone. The interpretation of the experimental data which is offered here is decidedly new, but the conclusion as applied to human arteriosclerosis does not by any means constitute an original suggestion. Thus, the real contribution of the experiments is that they provide new and strong support for an old and well founded idea.

In the human being there are fewer opportunities than could be desired to observe the effects of various arterial injuries of known origin and of a kind which might be expected to produce lesions capable of comparison with those of ordinary arteriosclerosis. The best example at one's disposal is the injury produced by syphilis of the aorta. It is well known, of course, that syphilis affects primarily the adventitia and media of the aorta, but in addition, the intima is secondarily injured. As a result of this injury, intimal lesions develop which may simulate very closely those of arteriosclerosis. This is most often the case when the syphilitic aortitis is not too recent in origin; that is, when the injury has persisted for a sufficient length of time to permit the relatively slow process of lipoid deposition in

the intima to become apparent. Such lesions, like those of ordinary arteriosclerosis, are characterized by a fibrous thickening of the intima and by more or less abundant accumulations of lipoids which are found partly in association with the intercellular material in the thickened intima and partly within foam cells.

Occasionally cases are seen at autopsy in which such "arteriosclerotic" lesions are found in the intima overlying the portions of the aortic wall which are affected by syphilis, while the remaining parts of the aorta and the other arteries throughout the body are practically free from arteriosclerosis. Evidently the injury to the wall of the vessel in these cases is responsible for the development of the arteriosclerotic changes associated with the syphilitic aortitis, for the uninjured parts of the aorta and the arteries elsewhere remain unaffected, although they are exposed to identical conditions so far as possible changes in the blood are concerned. Apparently, then, in the human being a suitable local injury to the intima is capable of producing intimal lesions which possess the essential characters of ordinary arteriosclerosis. The occurrence of injury, which is the primary event, is followed subsequently by the precipitation of lipoids and by cellular proliferation in the injured intima. Probably the same is true of arteriosclerosis as it occurs quite apart from injuries of such well recognized origin.

The cause of the arterial injury which initiates the development of human arteriosclerosis is obscure, and the cholesterol feeding experiments provide hardly any suggestive information bearing on this problem. In the past it has generally been taken for granted, on the basis of the experimental results, that hypercholesteremia in man has the ability to injure the arteries, but it has been shown here that the experimental data fail to demonstrate that this is true even in rabbits. The question of the real cause of the primary injury to the arteries in experimental cholesterol arteriosclerosis has never been carefully investigated. The evidence obtained in human beings, far from incriminating hypercholesteremia as a cause of arterial injury, indicates rather that hypercholesteremia of itself has no damaging effect on the arteries.

Most of the forms of experimental injury which have been employed as accessories to cholesterol feeding in rabbits seem to have no direct significance as applied to arteriosclerosis in man. The only exception of possible importance is the arterial injury produced in rabbits by intravenous injections of certain bacteria (see section VI *f* of this paper). It should be borne in mind, however, that the influence of bacterial infections on the development of experimental cholesterol arteriosclerosis is really not well established. The available experimental evidence bearing on this point is so meager that little importance can be attached to it at present. Moreover, caution must be exercised in the interpretation of such experiments, for it is well known that the

arteries of rabbits are extraordinarily susceptible to injury. Toxic agents which are injurious to the rabbit's arteries may not be equally injurious in man. Studies of human material should be more reliable, but they have led only to the formulation of a variety of opinions as to the rôle of infections in the etiology of human arteriosclerosis. It has been thought for a long time that bacteria, perhaps through the agency of their toxic products, may cause injuries to the arterial walls and thus may initiate the development of arteriosclerosis. This idea, although it has been favored by many students of arteriosclerosis, is difficult to prove, but is equally difficult to disprove. One can only say that the evidence which has been brought forward up to the present time is inconclusive.[97]

The tendency of the lesions of human arteriosclerosis to develop around the mouths of branching arteries is said to indicate that the injury at these points is due to the stretching and distorting action of mechanical forces. The lesions of experimental cholesterol arteriosclerosis likewise tend to favor the areas near the orifices of branching vessels and the same conclusion might be drawn from this fact, but the experimental observations really add nothing new to the arguments which have been built on observations on human arteries. In both instances, lesions are distributed in many places at a distance from possible weak spots in the walls of the arteries, so that it is difficult to believe that mechanical forces are wholly responsible. It seems more likely that the areas about the mouths of branching arteries are merely somewhat more susceptible than other parts of the wall of the vessel to the action of the injurious agent which produces lesions elsewhere. Whether this special susceptibility to injury is dependent on the operation of mechanical forces or on the peculiar structure of the wall of the vessel at such points or on some other local peculiarity remains undetermined. The experiments of Harrison,[59] of which some account has already been given, do suggest that mechanical forces which produce excessive internal movements within the arterial walls around the orifices of branching vessels are responsible for the tendency of lesions to develop in these areas.

Many other suggestions have been made as to the possible origin of the injury to the arteries which is responsible for the development of human arteriosclerosis. A discussion of these possibilities would be fruitless, however, for it is impossible at present to demonstrate that any one of the suggested causes of arterial injury is of decisive significance in the etiology of arteriosclerosis in man. One would suppose that the appropriate local injury must be one of moderate intensity which probably persists over a considerable period of time or which perhaps is repeated at intervals, but what it is that produces such an injury is really quite unknown.

The necessity of preliminary local alterations in the walls of vessels as a factor indispensable to the precipitation of lipoids and to the subsequent development of the lesions of human arteriosclerosis is perfectly clear, and there is every reason to believe that injury to the walls of the arteries can produce these changes. However, Aschoff [18] especially has emphasized the importance of primary alterations in the arterial walls which seem to occur simply as a part of the process of aging and which he believes are manifested chiefly in the intercellular cement substance or ground substance. Wells [175] has placed great emphasis on the deterioration of elastic fibers, a change which he has attributed to "aging" of the colloidal elastin of which these fibers are composed. Such alterations in the arterial walls undoubtedly occur in old age, but it is difficult to believe that the process of aging plays a part of any consequence in the development of arteriosclerosis in relatively young people. The importance which one can attach to the changes attributable merely to chronological old age limits itself automatically to the later decades of life. One can say only that the appropriate local alterations in the arterial walls, if they are not produced prematurely by local injury, will come eventually as a manifestation of age. So far as aging is a biologic property inherent in the human organism, it must be inevitable, and its consequences, therefore, are overshadowed in importance by those for which some form of injury to the walls of the arteries can be held responsible. Accordingly, the question of the cause or causes of this arterial injury still presents itself for further investigation as the most important problem in the etiology of human arteriosclerosis.

Attention may now be directed again to the lipoids which accumulate in the lesions of arteriosclerosis. The exact nature of the process by which lipoids come to be deposited secondarily in damaged areas in the walls of arteries has never been satisfactorily explained. The experimental observations indicate that changes in the intercellular ground substance are especially important in this connection, but no information is forthcoming as to the intimate nature of these alterations beyond the fact that they can be produced by injury. Neither is there any information available as to the manner in which the local changes can effect the precipitation of lipoids from the colloidal state in which they enter the walls of the arteries with the nutritive fluid. It is said that the lipoids are adsorbed by the altered intercellular material, but all of this is really obscure, a fact which emphasizes the necessity for further studies directed toward the clarification of this complex process.

Following the original injury to the wall of the vessel, when lipoid deposits begin to make their appearance, it becomes a question whether or not the lipoids in the intima can themselves act as injurious agents and thus propagate the arteriosclerotic process. It is probable that the original injury continues to act for a time at least, and the same injury

may be fully responsible for the whole development of the lesions, while the precipitation of lipoids in the injured intima may be entirely incidental. However, it seems highly probable that the precipitated lipoids, and especially the cholesterol esters which are apparently the most difficult of removal, play a part, and perhaps an important part, in the subsequent development of the arterial lesions. It is clear from the study of both the experimental and the human material that the lipoids deposited in the injured intima stir into activity the numerous macrophages which are attracted to the site and which then engulf a large part of the lipoid material. It seems entirely probable, too, that the free lipoid deposits can stimulate the proliferation of fibrous connective tissue cells in the intima. This effect is added to that of the original injury to produce a reparative fibrous tissue reaction in the affected areas. If the original injury which initiated the whole process ceases to operate, it is probable that the lipoid deposits prevent immediate healing and continue to exert their influence so that the lesions persist, progressing slowly. If this is true, the presence of lipoid deposits really renders the lesions of arteriosclerosis self-propagating. However, the experimental evidence indicates that the lipoids may be slowly removed under certain conditions, among which the withdrawal of the source of arterial injury is probably the most important. One can infer, therefore, that in human arteriosclerosis the termination of the influence of the original arterial injury may permit a gradual absorption of the lipoid deposits which, if they are not already too extensive, may even disappear finally, leaving only a fibrous thickening of the intima.

XI. SUMMARY AND CONCLUSIONS

Arterial lesions which resemble those of human arteriosclerosis can be produced in rabbits by the administration of diets containing considerable quantities of cholesterol. I have chosen to call this experimental disease of the arteries "experimental cholesterol arteriosclerosis." The literature bearing on experimental cholesterol arteriosclerosis is reviewed in detail. The results of attempts to produce arterial lesions by cholesterol feeding in animals other than rabbits are described. The data which have arisen from all of these experiments are summarized briefly in section VII of this paper.

On the basis of these data, the etiology and pathogenesis of experimental cholesterol arteriosclerosis in the rabbit are discussed. It is concluded that the presence of considerable quantities of cholesterol in the diet with a resulting elevation of the level of cholesterol and other lipoids in the blood is essential to the development of the typical arterial lesions in rabbits. It is shown, however, that hypercholesteremia alone cannot be regarded as the cause of the lesions in the arteries. There are preliminary local alterations in the walls of the arteries which pre-

cede the precipitation of lipoids. Evidence is advanced to show that these preliminary changes are due to some form of injury to the arteries attendant on the experimental procedure of cholesterol feeding. It is concluded that the occurrence of local changes in the arterial walls, due in all probability to injury of some kind, is the primary event in the development of the lesions of experimental cholesterol arteriosclerosis, an event which is followed subsequently by the precipitation of lipoids in the injured areas.

As a preliminary to the discussion of the significance of the experimental results, a comparison is drawn between the anatomic lesions of experimental cholesterol arteriosclerosis and those of human arteriosclerosis. It is demonstrated that the two diseases are not identical, and that there are a number of important differences between them. These differences are of such a nature as to suggest strongly that the rôle of the lipoids in the development of arteriosclerosis is greatly exaggerated in experimental cholesterol arteriosclerosis as compared with arteriosclerosis in man. A number of normal differences between the rabbit and man which render interpretation difficult and uncertain are pointed out. It is shown that the experimental data are not capable of accurate interpretation without recourse to data on corresponding points derived from the study of human material. With this fact in mind, an attempt is made to correlate the data concerning experimental cholesterol arteriosclerosis with the available information regarding human arteriosclerosis. The three main factors which can be recognized in the etiology of experimental cholesterol arteriosclerosis, namely, cholesterol in the diet, hypercholesteremia and injury to the arteries, are considered in order as regards their possible significance in the etiology of human arteriosclerosis. With full cognizance of the inferences which have been drawn in the past from the experimental results, the following conclusions are reached for reasons which are given in their appropriate places:

The results of cholesterol feeding experiments in rabbits do not constitute a valid reason for believing that an excess of cholesterol in the diet plays any rôle in the etiology of arteriosclerosis in man. No convincing evidence in support of such a belief exists at present.

Hypercholesteremia is not found with any regularity in association with human arteriosclerosis. It seems highly probable that arteriosclerosis in man can and usually does develop without deviation of the cholesterol content of the blood beyond the normal limits of variation. In any event, there is no valid evidence to support any other conclusion.

Hypercholesteremia of itself cannot be regarded as a cause of human arteriosclerosis. Although hypercholesteremia, when it occurs, might be expected on theoretical grounds to accelerate the development of

arteriosclerotic changes which have already been initiated, there is little evidence at present to indicate the existence of such an effect.

The cholesterol feeding experiments provide no valid reason for believing that a disturbance of cholesterol or lipoid metabolism plays any part in the etiology of human arteriosclerosis. There is no definite or concrete evidence from observations on human beings to support the idea. If a disturbance of lipoid metabolism participates in the etiology of arteriosclerosis in man, the fact remains to be demonstrated in the future.

The initial stage in the development of human arteriosclerosis consists of local changes in the walls of the arteries themselves, changes which are responsible for the subsequent precipitation of lipoids in the affected areas. In man, as in the rabbit, there is every reason to believe that these changes follow as the result of some sort of injury to the arterial walls. As to the cause of this injury, the experimental data yield hardly any information which is capable of application in the human being. Some possible causes of the arterial injury which have been suggested previously and on which the experimental results have some bearing are discussed briefly. Brief comment is also made on the influence of age. No definite conclusions are reached, since it is clear that the cause of the injury to the arteries which is responsible for the development of human arteriosclerosis is unknown.

Following the initial local injury, lipoids are deposited in the injured intima, especially in the intercellular substances. These deposits stir into activity numerous macrophages which are attracted to the site and which then engulf a large part of the lipoid material. It seems entirely probable that the free lipoid deposits can stimulate the proliferation of connective tissue cells in the intima. This effect is added to that of the original injury to produce a reparative fibrous tissue reaction in the affected areas. If the original injury which initiated the whole process ceases to operate, it is probable that the lipoid deposits prevent immediate healing and continue to exert their influence so that the lesions persist, progressing slowly. Under these conditions, however, it seems likely from the experimental evidence that the lipoids may be slowly removed and may even disappear finally, leaving only a fibrous thickening of the intima. It is unnecessary to assume that a disturbance of cholesterol or lipoid metabolism plays a part in any stage of the process.

This outline of the development of human arteriosclerosis is entirely consistent with the knowledge derived from the study of arteriosclerosis in man, and at the same time it incorporates those inferences which can be drawn with justification from the experimental data. It seems to be the most reasonable working hypothesis which can be constructed from the evidence available.

A tentative scheme of the relationship between experimental cholesterol arteriosclerosis and human arteriosclerosis is offered which explains in a logical way the apparent discrepancies between the conclusions which have been drawn in this paper regarding the two diseases.

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Notes and News

Society News.—The Third International Congress of Comparative Pathology will be held in Athens, Greece, on April 15 to 18, 1936. Special reports will be made on nephrosis, amyloidosis, leishmaniasis, spirochetosis and avitaminosis. Information may be obtained from Prof. Anthony Codounis, 40 Didotou Street, Athens.

Grants in Aid of Research.—Applications for grants in aid of research for 1936 by the American Association for the Advancement of Science must be received at the permanent secretary's office in Washington, D. C., on or before October 30 next.

The Division of Medical Sciences of the National Research Council will hold a special meeting in November 1935 for the consideration of applications for grants-in-aid in this field. Applications to be considered at this meeting must be on file with the secretary of the committee on grants-in-aid, Dr. Clarence J. West, not later than Oct. 1, 1935. Applications received after Oct. 1 and prior to Feb. 15, 1936, will be acted on at the next regular meeting of the committee in March 1936.

Resolution in Regard to the Coroner's Office.—At the annual meeting of the Illinois State Medical Society, on May 21 to 23, 1935, the following resolution was adopted by the house of delegates:

WHEREAS, the Coroner's duties are both magisterial and medical, each of these requiring a high degree of specialized knowledge; and

WHEREAS, the Coroner has no qualifications except political ability to get votes, not being required to be either lawyer or doctor; and

WHEREAS, the Coroner does nothing that must not be done over again; and

WHEREAS, There are grand and petit juries to handle the work of the Coroner's jury; and

WHEREAS, The office of Coroner is both useless and costly; and

WHEREAS, The press is heartily in sympathy with this move as evidenced by editorials such as "The Office of Coroner" (*Chicago Daily News*, April 7, 1933); "What Price Gangster Protection" (*Chicago Daily News*, Oct. 7, 1933); "An Outrageous Verdict" (*Chicago Daily Tribune*, April 1933), and many other editorials and news stories appearing from time to time;

BE IT THEREFORE RESOLVED, That the Illinois State Medical Society go on record as favoring the abolition of the Coroner's office and establishing in its stead a Medical Examiner who is a licensed doctor of medicine, to be appointed by the governor or civil service commission, whose duties will be medical examination only to determine causes of death, the legal investigative procedures to be left to the district or states attorneys;

AND BE IT FURTHER RESOLVED, That copies of this resolution be sent to the Illinois State Bar Association and to the Press.

Society Transactions

BUFFALO PATHOLOGICAL SOCIETY

Regular Meeting, Jan. 23, 1935

KORNEL TERPLAN, *President, in the Chair*

W. F. JACOBS, *Secretary*

THE FIBER AND NUCLEAR STRUCTURES IN A CASE OF HYPOPLASIA OF THE CERE-
BELLUM. GILBERT BECK.

A 9 months old child died after the making of an encephalogram. The cerebrum
was normal in size, cell and fiber structure. The cerebellum, on the other hand,
was found to be long and narrow, though it contained the normal number of
lobules and sulci. It resembled the cerebellum in miniature described by Nonne.
Serial sections were made of one half of the cerebellum in the transverse direction
and of the other half in the sagittal plane and stained by the Weigert-Pal method.

Cerebellum Proper.—The myelin of both the vermis and the flocculus was well
stained. The lobules of the hemisphere, however, were myelinated in varying
degrees. The tonsilla was most poorly myelinated, the biventer not so poorly,
while the myelin of the lobuli gracilis and quadrangularis was somewhat better
retained. The lobuli semilunares superiores and inferiores were as deeply stained
as the vermis.

The pars intermedia was confirmed. It demonstrated a connection between
the hemisphere and the emboliform nucleus.

Brain Stem Nuclei and Tracts Associated with the Cerebellum.—The nuclei
arcuati and the stria Piccolomini were absent. The inferior olive and the
accessory olives were normal. Fibers from these structures could be traced swing-
ing above the dentate nucleus into the lobuli semilunares. This is contrary to
Holmes' and Stewart's schematic representations as well as Brouwer's conclusions.

All pontile nuclei other than the peripeduncular, intrapeduncular and reticular
groups were absent. The stratum superficialis pontis as well as the fibrae rectae
pontis were well retained. This seems to prove that this group of fibers and
the reticular nucleus are more ancient than the rest of the arms of the pons.

Cerebellar Nuclei Proper.—(*a*) The dentate nucleus showed the macrogyric
ventral sheath more embryonic than the microgyric dorsal sheath. The latter
also was immature, giving few if any fibers to the superior peduncle.

(*b*) The emboliform, or frontal conglomerate, nucleus was composed of very
large cells and extremely dense fiber structures. This nucleus was the sole source
of origin of the dentatorubrothalamic pathway.

(*c*) The nuclei globosi and fastigii appeared normal.

Cerebellar Connections.—The middle peduncle with the exception of the stratum
superficiale was entirely aplastic.

The superior peduncle originated from the emboliform nucleus and decussated
in the brain stem to form the dorsal portion of the commissure of Wernekinck,
thus confirming Hatchek's experimental work. The restiform body and the ven-
tral spinocerebellar tract appeared normal.

A definite and unmistakable connection between the mesencephalic trigeminal
root and the cerebellum was confirmed; in sagittal sections many fibers could be
traced emerging from the trigeminal root into the cerebellum.

It seems that the hypoplasia of the cerebellum may have been due to a lack of proper neurobiotaxic stimulation because the arm of the pons was not normally developed. Two conditions are offered in confirmation of this view:

(*a*) Fetal cerebral atrophy causes contralateral cerebellar atrophic changes, whereas atrophy of the adult cerebrum (Pick's disease, porencephaly, etc.) in most case does not cause these changes. Were it just a matter of the amount of fibers, a difference between "secondary" changes following fetal disturbances, on the one hand, and atrophy of the adult cerebrum, on the other, would not be present. In fetal life the contralateral atrophy must be regarded in the light of a lack of the proper neurobiotaxic stimulation for "massive" development.

(*b*) In the semilunar lobes, in which the myelination was very splendidly retained through the olivocerebellar fibers, the lobules were, however, still very small, no larger than the adjacent lobules, in which the amount of myelin was scant. The well myelinated semilunar lobules also lacked the neurobiotaxic stimulation necessary for their development in the large, "fleshy" lobules of the hemisphere that one knows of normally.

Regular Meeting, Feb. 16, 1935

Kornel Terplan, *President, in the Chair*

W. F. Jacobs, *Secretary*

Demonstration of Sucrose in the Cerebrospinal Fluid of a Patient with Acute Encephalitis. Roger S. Hubbard and Kornel Terplan.

The effect of sucrose injected intravenously on the pressure of the cerebrospinal fluid has recently been described (Bullock, L. T.; Kinney, R., and Gregerson, M. I.: *Am. J. Physiol.* **109**:17, 1935). When concentrated solutions of the sugar are injected intravenously a fall in intracranial pressure occurs. Later there is a gradual return to normal values without the secondary rise which is sometimes noted when dextrose is used.

In the course of the treatment of a patient with encephalitis 250 cc. of a 26 per cent sterile solution of the sugar was injected to decrease the intracranial pressure. The patient was a 7 year old boy who had enjoyed perfect health until about three weeks prior to his death, when a cold in the head with severe cough developed. Although there was no whooping or vomiting, two doses of therapeutic pertussis vaccine were given. Only in the last three days of life the temperature rose to 101 F. The boy felt chilly and complained of headache and dizziness. General convulsions developed, followed by deep coma. There was slight rigidity of the neck with a positive Babinski sign on the right side. The spinal fluid showed 56 cells but was clear. The child died showing symptoms of pulmonary edema six hours after the intravenous injection of sucrose. The clinical diagnosis was "acute encephalitis (?)".

The autopsy was performed six hours after death. The brain showed unusually marked swelling and hyperemia; its weight was 1,400 Gm. The amount of spinal fluid was distinctly decreased. Only 7 cc. was collected from the basal cisternae. The ventricles contained very little fluid; they appeared markedly compressed by the swollen brain substance. Except for acute catarrhal tracheobronchitis, inflammatory edema of the lungs and recent lobular pneumonia there were no other noteworthy findings.

Histologically the brain showed an almost diffuse inflammatory process involving the white and the gray matter alike. There were moderately dense perivascular lymphocytic infiltrates, especially around small veins. In a few areas, e. g., the caudatum and the frontal lobe, the inflammatory process showed focal accentuation with small nodular infiltrates. The leptomeninges, too, showed slight focal infiltration with lymphocytes. The pons, the medulla oblongata and the upper part of the cervical cord were practically free from such infiltrations.

It seemed worth while to determine whether sucrose could be demonstrated in the fluid obtained at the postmortem examination. For this purpose a specimen of the fluid was treated with hydrochloric acid to give a concentration thirty-three hundredths normal and was then heated on a water bath for one-half hour to hydrolyze any disaccharide which might be present. The acid was then neutralized and the levulose determined by the method of J. H. Roe (*J. Biol. Chem.* **107**:15, 1934). A control specimen obtained before the sucrose was injected was also treated in the same way, and the levulose content of both specimens was determined without submitting them to acid hydrolysis. The results of these analyses are given in the table.

Apparent Levulose Content of Spinal Fluid From a Patient with Encephalitis

Specimen	Milligrams of Levulose per 100 Cc.	
	Before Injection of Sucrose	After Injection of Sucrose*
Hydrolyzed	3.4	15
Unhydrolyzed	3.4	10

* Specimen obtained after death.

The figures show clearly that the fluid taken at the autopsy contained a substance which yielded levulose on acid hydrolysis. This substance was absent from spinal fluid taken before sucrose was administered. It seems practically certain, therefore, that sucrose was present in the fluid. The concentration of sucrose can be calculated approximately from the formula:

$$(15\text{—}3.4) \times \frac{\text{molecular weight sucrose}}{\text{molecular weight levulose}} = 22 \text{ mg. sucrose per 100 cc. of fluid.}$$

Because Roe's method gives positive results with unhydrolyzed sucrose as well as with levulose, and because spinal fluid contains an unexpectedly high concentration of levulose (Hubbard, R. S., and Garbutt, H. R.: *Proc. Soc. Exper. Biol. & Med.* **32**:986, 1935), further experiments were carried out in an attempt to devise a more satisfactory method for the determination of sucrose in the spinal fluid. It was found possible to do this by incubating spinal fluid with one of the strains of Bacterium coli which ferments fructose but does not attack sucrose, subjecting the incubated material to acid hydrolysis and carrying through Roe's procedure. When this technic was applied in further experiments the entrance of sucrose into the spinal fluid after the injection of the sugar into the blood stream was confirmed.

Regular Meeting, March 27, 1935

Kornel Terplan, *President, in the Chair*

W. F. Jacobs, *Secretary*

Some Immunologic and Serologic Problems in Tuberculosis. Ernst Witebsky, Heidelberg and New York (by invitation).

While diseases such as scarlet fever are followed by definite immunity against reinfection, it is doubtful whether immunity against chronic infectious diseases such as tuberculosis and syphilis exists. Since the time of Robert Koch it has been known that a tuberculous animal will react to a second infection (reinfection) differently from a normal one. A lesion will appear as early as a few hours after reinfection. It vanishes a few days later and is not followed by a general infection. This typical behavior, also called "infectious immunity," is due to the presence of an active tuberculous lesion in the body. "Infectious immunity" means immunity during the stage of actual infection. The "infectious immunity"

in tuberculosis can be compared to that in syphilis, in which reinfection does not induce a primary lesion at all. The failure of the syphilitic person to acquire a primary lesion is due to the presence of living spirochetes somewhere in the body. After complete cure by antisyphilitic treatment, however, it is possible again to produce a primary lesion as in noninfected persons.

Regarding the striking similarity between the Koch effect on the one hand and the Arthus phenomenon on the other, many authors maintain that the tuberculin test is based on an antigen-antibody reaction. However, there are important differences between both. It is possible to transfer the Arthus phenomenon by means of the serum of a sensitized person to a normal one. The attempt at passive transfer of the hypersensitiveness against tuberculin fails. Neither does tuberculin (the bacteria-free filtrate of cultures of tubercle bacilli) induce the formation of antibodies, nor is it possible to prove the existence of antibodies against tuberculin in the serum by precipitation reactions or complement fixation. Thus, tuberculin lacks all the qualities which characterize an antigen. In spite of recent important investigations carried out by Seibert and by Long concerning the chemical nature of tuberculin it must be stated that the basis of the tuberculin reaction is still a mystery of nature.

The experimental analysis of serum in tuberculosis shows two different changes: (1) an alteration of the albumin-globulin ratio; (2) the presence of specific antibodies. Many reactions are described which are based on the increased lability of plasma and serum in tuberculosis. Reagents such as alcohol, saline solutions, distilled water and some chemical reagents are used. The increase of lability sometimes parallels the intensity of the actual process. The change in the albumin-globulin ratio is, however, not specific for tuberculosis but occurs also in such conditions as carcinoma, acute infectious diseases and pregnancy. The presence of real antibodies in the serum of tuberculous people may be demonstrated by using specific antigens only, e. g., tubercle bacilli or certain substances derived from tubercle bacilli. By means of the specific absorption test it is possible to prove the presence of antibodies in the serum of patients suffering from tuberculosis. During the last few years several new antigens and reactions for the serodiagnosis of tuberculosis have been described, based mostly on complement-fixation methods. Their sensitivity as well as specificity has improved very much. The experience derived from these methods shows that, especially in adults suffering from phthisis of the lungs, a high percentage (about 80 per cent) of positive results is obtained. On the other hand, in the so-called second Ranke's stage of tuberculosis, e. g., tuberculosis of the skin, bones, joints and peritoneum, the percentage of positive reactions obtained is rather low (25 per cent or less). The complement-fixation test in tuberculosis may be of differential diagnostic value when antigens the specificity as well as the sensitivity of which is satisfactory are employed. The test is also suitable for the examination of pleural fluids and may be of great help in deciding the question whether or not a pleural fluid is tuberculous. It is difficult thus far to make a statement concerning the prognostic value of the presence of antibodies in the serum in tuberculosis. A rather high percentage of serums from dead bodies contain antibodies. Thus they do not necessarily inhibit the progress of the disease. Further investigations are necessary if one is to answer the question whether the presence of antibodies after successful pneumothorax treatment is due only to the persistence of antibody functions or to the fact that tubercle bacilli are still present somewhere in the body.

Tuberculin reactions, lability reactions and antigen-antibody reactions in tuberculosis are independent of each other. Although the definite interpretation of these biologic tests is still a subject for further study, their suitable application may already be of value for clinical purposes.

ISOLATION OF A TUBERCULIN-LIKE SUBSTANCE FROM THE URINE OF PATIENTS WITH ACTIVE TUBERCULOSIS AND ITS PRACTICAL APPLICATION AS AN AID IN DIAGNOSIS. E. B. HANAN.

These studies are based on the principle that a tuberculin-like substance of specific antigenic nature is excreted in the urine during the active stage of tuber-

culosis.   When concentrated and partially purified this material may be used in
conjunction with old tuberculin as an aid to distinguish between the latent and
the active phases of the disease. Vacuum-concentrated and dialyzed urinary extracts
were obtained in 124 cases of tuberculosis.   By the Mantoux technic, 0.1 cc.
of the extract was injected near the site of a similar injection of a 1 :10,000 dilution
of old tuberculin on the forearm of the patient from whom the urine was obtained.
In 107 of these cases active tuberculosis was proved to be present.   The reaction
to old tuberculin was questionable in 7 and negative in 9. Seventeen patients were
proved not to have tuberculosis.   The old tuberculin provoked no reaction in 10;
the auto-urinary extract, in 15.   Twenty normal persons gave negative reac-
tions to auto-urinary extract.

In further studies it was observed that a chloroform-ether mixture extracted
from a water solution, slightly alkaline or acid in reaction, one tenth of a known
amount of ultraprotein tuberculin.   This method provided a more simple pro-
cedure than the vacuum concentration and dialyzation method.   A modification
using chloroform-ether extraction was applied as a routine in 166 cases of
suspected tuberculosis.   In 44 of the cases the disease was proved to be active
tuberculosis.   Old tuberculin gave a negative result in 1 and the auto-urinary
chloroform-ether extract a negative result in 5. In 64 of the cases active tuber-
culosis could not be proved.   The reaction of old tuberculin was negative in 51
and positive in 13.

These results were not entirely gratifying but indicate the importance of this
kind of investigation. Efforts were made to isolate the active principle in a more
highly concentrated and purified form in order to test its specific antigenic nature.
In studies on tuberculin precipitation it was observed that the optimum hydrogen
ion concentration at which ultraprotein tuberculin precipitated was p_H 2.8. It was
further observed that when urine from persons with active tuberculosis was
adjusted to p_H 2.8 turbidity frequently developed.   This reaction was not
specific, but when it occurred in a case of tuberculosis the turbidity was noted to
increase in relation to the severity of the disease.   Urine showing marked turbidity
was collected from a tuberculous patient to the amount of 1,000 cc., and the
tuberculin-like substance was concentrated and purified by a benzoic acid method.
This material proved to be highly antigenic, giving specific tuberculin reactions
and specific fixation of complement with serums from patients with active tuber-
culosis and from immunized rabbits.

Further studies are being carried out on the isolation of this tuberculin-like
substance from urine by absorption methods.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

THE COURSE OF RHEUMATIC HEART DISEASE. A. C. DeGRAFF and C. LINGG, Am. Heart J. **10**:459, 1935.

The course of rheumatic heart disease is described as observed in 644 patients who are dead in a total of 1,633 patients coming under observation in the ten years up to 1931. Of these patients 55.8 per cent were males and 44.2 per cent were females. Rheumatic heart disease usually existed alone (94.5 per cent) and was seldom combined with other etiologic types (5.5 per cent). This disease was in the main one of childhood and early adult life, for it occurred and ran its course chiefly within the first four decades of life. The proportion of sufferers more than 40 years of age was small. Three-fourths of those who survived to adult life were dead before the age of 40. The average age at the time of the initial infection was 17 years; that at the time of the first symptoms of cardiac insufficiency was 28 years; that at the first appearance of heart failure was 30 years, and that at death was 33 years. Stated in another way, the average patient was infected at the age of 17 but was free from symptoms and able to carry on ordinary physical activity for eleven years. He then began to suffer from diminished cardiac reserve culminating in heart failure two years later. From this time on until death three years later he was wholly an invalid or at least, in most cases, seriously incapacitated. The period of economic usefulness of the person afflicted with rheumatic heart disease was, on the average, not more than eleven years after the initial rheumatic infection; in most cases it was less than nine years. Once symptoms of cardiac insufficiency appeared heart failure and death followed rapidly. Fifty per cent suffered their first symptoms and failure and died within a period of from sixteen to twenty years, or between 20 and 40 years of age, the years of early maturity. To see even terminal stages of this disease after the age of 50 years was not a common experience. Death usually occurred as the result of heart failure, but life was shortened in a fair proportion of cases by such conditions as subacute bacterial endocarditis, pneumonia and other diseases. AUTHORS' SUMMARY.

LATENT CARDIAC COMPLICATIONS FOLLOWING SYDENHAM'S CHOREA. H. SCHWARZ and S. D. LEADER, Am. J. Dis. Child. **49**:952, 1935.

Seventy-five cases of so-called pure chorea were observed for from one to twelve years for evidence of cardiac involvement. The term "pure" is used to describe chorea in which no other manifestations of rheumatism were noted clinically. Cardiac involvement was diagnosed by physical signs, roentgenograms and electrocardiograms. The roentgenographic evidence was remarkably constant and corresponded well with the physical findings; occasionally, positive roentgenographic evidence was found in cases in which the physical findings were of no significance. The longer the period of observation after the first attack of chorea the higher was the percentage of cardiac involvement; after seven or eight years the percentage apparently approached 100, although the number of cases studied is too small to permit a definite statement. The signs of cardiac involvement developed insidiously, without recognizable attacks of rheumatic fever and in some cases without further attacks of chorea. In this series aortic murmurs, pericarditis and subcutaneous nodules were never encountered. It seems probable that what is said of rheumatic fever may also be said of chorea: "The heart is always involved." This probable cardiac involvement is added evidence that Sydenham's chorea is of rheumatic origin. FROM THE AUTHORS' SUMMARY.

HEMORRHAGIC ENCEPHALITIS. A. B. BAKER, Am. J. Path. **11**:185, 1935.

Twenty fatal cases of encephalitis with hemorrhagic changes in the brain are described. In one case the brain tissue was virulent on inoculation in the brains of rabbits. Baker believes that hemorrhagic encephalitis is a distinct disease.

ANTIGROWTH EFFECT OF LIPOID FRACTIONS OF TISSUE EXTRACTS. F. A. McJUNKIN and J. W. HENRY, Am. J. Path. **11**:353, 1935.

The lipoids of organs exert an antigrowth effect on the kidney and liver on injection into young rats. The action is potent, since the quantity contained in 0.5 Gm. of fresh kidney is effective; the inhibiting substance is probably in the phospholipid fraction; it is not specific, is not limited in its action to one or more organs and is widely distributed in the body. An explanation of the function of lipoids in the regulation of cell growth must await investigation of other factors concerned in nuclear division.

THE CEREBROSPINAL FLUID IN EXPERIMENTAL POLIOMYELITIS OF MACACUS RHESUS. MAURICE BRODIE and BERNARD WORTIS, Arch. Neurol. & Psychiat. **32**:1159, 1934.

Brodie and Wortis studied the spinal fluid of over one hundred rhesus monkeys experimentally inoculated with poliomyelitis virus and compared their observations with those on the spinal fluid of normal monkeys. In the early stages of the disease the spinal fluid usually showed predominance of polymorphonuclear cells; with the onset of paralysis there was an excess of lymphocytes. In some cases the cell count was normal, and it was not as a rule influenced by the stage of the disease (preparalytic, paralytic or extreme paralytic). In the mild, nonparalytic type the cell count became normal in from three to ten days, and in severe forms, within the fourth week after the onset of the paralysis. Pleocytosis developed in from twelve to thirty-six hours after an incubation period of from three to seven days followed by a rise of temperature (2 to 6 F.). Symptoms of involvement of the central nervous system appeared either immediately or within forty-eight hours or even later. An important pathologic observation was that though the meninges were involved the primary lesion was in the spinal cord. In general, the clinical types of experimental myelitis and their serologic features were similar to those observed in man. The respiratory quotient for animals intracerebrally inoculated with poliomyelitis was found to be normal— the spinal cord of such animals was able, for instance, to oxidize dextrose.

GEORGE B. HASSIN.

CHANGES IN THE CEREBRAL CORTEX PRODUCED BY THERMOCOAGULATION. J. G. DUSSER DE BARENNE and H. M. ZIMMERMANN, Arch. Neurol. & Psychiat. **33**:123, 1935.

Dusser de Barenne demonstrated that thermocoagulation of the cortex causes local destruction of the ganglion cells of the superficial lamellae. In acute states, with a survival period of from seven to twelve days, only the ganglion cells are destroyed; in chronic experiments, with the application of a temperature of 80 C. and a survival period of four months, the glial and mesodermal elements also are destroyed. Aside from the time element, the degree of heat used is of importance. Thus, a temperature of 65 C. applied locally to the cortex for two seconds causes destruction of two outer layers; applied for three seconds, it causes additional destruction of a portion of the third layer. A temperature of 70 C. applied for three seconds destroys three outer layers and when applied for four seconds affects four outer lamellae. Application of a temperature of 50 C. for half a minute also produces destruction of the ganglion cells, but there are marked reactive proliferation of the glial and fibroblastic elements of the upper three lamellae and preservation of some pyramidal cells of the deeper portion of the

third layer. As chronic thermocoagulation of 80 C. produces in contrast complete devastation of the thermocoagulated cortical tissues without subsequent postoperative irritation, the authors suggest thermocoagulation as a method of treatment for cortical epilepsy when excision of cortex is indicated.

GEORGE B. HASSIN.

HYPOTHERMIA IN HYPOTHALAMIC LESIONS. C. DAVISON and N. E. SELBY, Arch. Neurol. & Psychiat. **33**:570, 1935.

A man aged 31 presented adiposogenital dystrophy combined with polydipsia, polyuria and prolonged hypothermia. The average temperature for three months was 93 F.; the minimum (rectal) was 90.7 F.; the maximum, 96.6 F. Immersion in a hot water bath (108 F.) caused a rise in the oral temperature to 100.1 F.; immersion in a cold bath, to from 93.2 to 94.2 F. Necropsy revealed a hemorrhagic tumor (angioma) between the floor of the third ventricle and the sella turcica. It extended from the optic nerve to the mamillary bodies, enclosed the chiasm and optic tracts and destroyed, among other structures, many hypothalamic nuclei but spared the pituitary body. The nuclei of the tuber cinereum were destroyed completely, while the vegetative nuclei in the region of the mamillary body were only partly destroyed. The partial destruction of the paraventricular and supra-optic nuclei is thought to have been the cause of the slight polyuria and polydipsia.

GEORGE B. HASSIN.

THE EFFECT OF HYPOTHALAMIC LESIONS AND STIMULATION OF THE AUTONOMIC NERVOUS SYSTEM ON CARBOHYDRATE METABOLISM. L. DAVIS, D. CLEVELAND and W. R. INGRAM, Arch. Neurol. & Psychiat. **33**:592, 1935.

The authors studied carbohydrate metabolism in cats in which they had damaged the pancreas, hypothalamus and sympathetic nerves. They used the Horsley-Clarke stereotaxic instrument to make lesions in the hypothalamic region with precision. Removal of the pancreas in hypophysectomized cats does not produce hyperglycemia or glycosuria, but in those with a lesion of the tuber cinereum it was followed by pancreatic diabetes. In a series of cats hypothalamic lesions were made first and the pancreas was removed some weeks later. Temporary hyperglycemia followed the bilateral, symmetrical destruction or irritation of the hypothalamus, and in such animals pancreatectomy also caused temporary hyperglycemia followed by normal metabolism of sugar. Stimulation of the superior cervical sympathetic ganglion or of the stellate ganglion in cats produced hyperglycemia and glycosuria, which were not present in animals in which the splanchnic nerves had been removed.

GEORGE B. HASSIN.

CORTICOSPINAL FIBERS ARISING IN THE PREMOTOR AREA OF THE MONKEY. E. C. HOFF, Arch. Neurol. & Psychiat. **33**:687, 1935.

By studying the degeneration of terminal buttons (*boutons terminaux*) in the spinal cord following experimental lesions of the premotor area in monkeys Hoff could demonstrate that this area gives rise to a separate system of corticospinal fibers. The degeneration of the terminal buttons in the spinal cord is bilateral, is usually limited to the base of the dorsal horns, and is predominantly in the contralateral side. One group of the corticospinal fibers passes superficially from the premotor into the motor area. Hoff has thus furnished histologic proof that, in addition to the motor area, there is a premotor area with a separate group of corticospinal fibers.

GEORGE B. HASSIN.

CORTICOSPINAL FIBERS ARISING IN THE PREMOTOR AREA OF THE MONKEY. M. A. KENNARD, Arch. Neurol. & Psychiat. **33**:698, 1935.

Kennard studied with the method of Marchi the system of so-called nonpyramidal corticospinal fibers. These originate in the so-called premotor area (Brod-

mann's area 6), ablation of which in monkeys invariably caused descending degeneration of these fibers. Their course is along with the pyramidal tracts to the lower lumbar levels of the spinal cord, contralaterally and ipsilaterally. The degeneration was especially heavy after extirpation of both the motor and premotor areas, but no degenerative fibers were found in the ventral columns after destruction of the premotor area alone.

GEORGE B. HASSIN.

THE HEALING OF ARTIFICIAL DEFECTS OF THE DUODENAL MUCOSA. H. W. FLOREY and H. E. HARDING, J. Path. & Bact. **40**:211, 1935.

Brunner's glands are found to regenerate, but on regeneration they occupy for the most part the region of the crypts of Lieberkühn instead of their usual submucosal site. The duodenal mucosa gives rise to the superficial type of gastric epithelium, which probably arises from Brunner's glands. The possible significance of this is mentioned.

FROM THE AUTHORS' SUMMARY.

THE PITUITARY GLAND IN ADDISON'S DISEASE. A. C. CROOKE and D. S. RUSSELL, J. Path. & Bact. **40**:255, 1935.

A reduction in the number of the basophil cells is considered to be a constant change and the most significant change in the other ductless glands following destruction of the adrenal cortex in Addison's disease. It is suggested that this reduction is the cause of the low blood pressure and possibly of the hypoglycemia in this disease.

HAEMOGLOBINOCHOLIA IN TOXIC CONDITIONS. R. MUIR and J. F. HEGGIE, J. Path. & Bact. **40**:335, 1935.

After administration of various poisons to rabbits hemoglobin in small amounts can be detected in the bile of the gallbladder. This occurs both with poisons which lead to hemoglobinemia and with those which have not this effect. The hemoglobin has been detected only by the Kastle-Meyer reaction, but when some time has elapsed after the death of the animal hemoglobin may be present in such amount as to give distinct spectroscopic bands. This is apparently the result of the action of the bile salts on the erythrocytes within the vessels of the gallbladder mucosa. In cases in which hemoglobin has been found in the bile in the gallbladder it has been detectable also in that of the common bile ducts; its origin is, at least in part, hepatic. These results with the negative results in pure hemoglobinemia indicate that the hemoglobinocholia is due to damage of the liver with escape of a few erythrocytes into the bile capillaries. Such damage in varying degree may be found in phosphorus poisoning but has not been demonstrated in other conditions in which hemoglobin occurs in the bile.

FROM THE AUTHORS' SUMMARY.

AUER'S BODIES IN MONOCYTIC LEUKEMIA. J. C. HAWKSLEY, J. Path. & Bact. **40**:365, 1935.

The significance of these bodies remains unexplained. They appear to be commoner in the more immature cells, and may be due to some rare abnormality in cell metabolism peculiar to leukemic cells. They are not pathognomonic of any type but are of doubtful occurrence in lymphocytic leukemia. It is possible that further investigation of the bodies may link certain cases of leukemia which at present appear not to be closely related.

CULTURES OF LEUKOCYTES IN VITRO. G. D. BELONOVSKY, Arch. internat. de méd. expér. **9**:367, 1934.

In tissue cultures of human blood from a single clot variations in the type of leukocytes predominating commonly occur. If transplanted frequently the cultures

can be kept alive for as long as three months. In cultures of blood from persons with active tuberculosis there is rapid formation of leukocytes, which die sooner than those from normal blood. Tuberculin added in small amounts to cultures destroys the cells of blood from tuberculous patients but shows little or no effect on normal blood cells. Cultures of leukocytes inoculated with virulent tubercle bacilli are protected, to some extent, by a previous inoculation with BCG. Cultures of leukocytes from patients with scarlet fever are also characteristic, showing an increase in eosinophils and many dead cells after forty-eight hours of cultivation. There is evidence that Bacillus coli may be transformed into Bacillus typhosus by cultivation in symbiosis with human cells. The effect of various mineral waters on cultures of leukocytes is being investigated.

RALPH FULLER.

RELATION OF HEALTH TO SOLAR ERUPTIONS. TRAUTE and B. DÜLL, Virchows Arch. f. path. Anat. **293**:272, 1935.

Traute and Düll review the literature dealing with the relation of human disease and death to meteorological conditions. They criticize the statistical methods used by medical writers in the analysis of meteorological data and the deductions drawn therefrom; they claim that medical writers are not sufficiently familiar with the newer knowledge of geophysics and astrophysics. Such correlation as has been found between weather conditions and disease and death is not proof of the dependence of the latter on the former; both are coordinate manifestations of solar phenomena. For the medical reader a discussion is presented of electronic invasion of the earth's troposphere during the sudden eruptions that occur in the so-called M regions of the sun's surface. The commonest manifestation of such electronic invasion is a disturbance of the earth's magnetic field. Thirty-six thousand deaths occurring in Copenhagen in the five year period from 1928 to 1932 and four thousand in Zurich were analyzed with reference to these solar phenomena. A regularly cyclic heaping of deaths every twenty-seven days was observed. From the analysis of the data it was possible to predict the time after periods of solar activity when deaths in various disease groups would increase. The effects of solar activity on the human organism are ascribed to the action of rays of characteristic wavelength emitted by the sun during periods of eruption.

O. T. SCHULTZ.

FORMATION OF BILIRUBIN IN TISSUE CULTURES OF SPLEEN. S. SÜMEGI, M. CSABA and E. VON BALOGH, Virchows Arch. f. path. Anat. **293**:320, 1934.

Previous work had led to the conclusion that the formation of bilirubin in tissue cultures of spleen is the result of vital activity of cells of the reticulo-endothelial system and not of an extracellular ferment as has been held by many. Further work on tissue cultures confirmed the authors' previous findings. Potassium cyanide, alterations in hydrogen ion concentration and toxins influenced the formation of bilirubin adversely only when they interfered with cellular activity and growth. Tissue of a spleen that had been subjected to reticulo-endothelial blockade before explantation grew in culture, but bilirubin formation was diminished because of the decreased vital activity of the blockaded cells. Bilirubin was not formed in sterile mixtures of blood and cerebrospinal fluid, contrary to others' findings which have been interpreted as evidence of extracellular ferment activity.

O. T. SCHULTZ.

EFFECT OF IONIZED AIR ON THE LEUKOCYTIC BLOOD PICTURE OF GUINEA-PIGS. W. N. NEKLUDOW ET AL., Virchows Arch. f. path. Anat. **293**:438, 1934.

An apparatus was devised by means of which positively or negatively ionized air at a uniform temperature could be delivered to a chamber in which guinea-pigs were kept. Short exposure to negatively or positively ionized air caused temporary leukopenia, monocytosis of short duration and transient increase in pseudo-eosin-

ophils. Prolonged exposure to negatively ionized air caused leukocytosis, chronic relative monocytosis, which is ascribed to activity of the reticulo-endothelial system, and decrease in the percentage of lymphocytes.   O. T. SCHULTZ.

BLUE SCLERA. J. HIRSCHMANN, Ztschr. f. klin. Med. **126**:718, 1934.

In four of six families with blue sclera there was defective development of bone, cartilage, ligaments, connective tissue and hematopoietic and lymphatic organs. Dominant inheritance of blue sclera in the male members of the family was shown in a pedigree of five generations.   FROM THE AUTHOR'S SUMMARY.

Pathologic Anatomy

LOCALIZATION OF CARDIAC INFARCTS ACCORDING TO COMPONENT VENTRICULAR MUSCLES. J. S. ROBB, J. G. F. HISS and R. C. ROBB, Am. Heart J. **10**: 287, 1935.

Certain coronary branches are "end arteries" to the individual ventricular muscle bands. If such end arteries are ligated the resulting infarcts affect the electrocardiogram consistently. Elimination of either or both of the superficial muscles alters the blood pressure little or not at all. Elimination of either of the deep muscles causes a marked fall in blood pressure, and if the lesion is large death is immediate. These results cannot be explained according to present-day conceptions of the physiology of conduction.

FROM THE AUTHORS' CONCLUSIONS.

PATHOLOGY OF CORONARY SCLEROSIS. T. LEARY, Am. Heart J. **10**:328, 1935.

From this study of the lesions of coronary sclerosis it is evident that the pathology of the disease as given in most textbooks needs to be rewritten. The pathology in these works is based on cases of coronary sclerosis in which the patients were hospitalized and in which secondary changes, including infarction with associated inflammatory reactions, tended to obscure the picture. The material for the present investigation came from patients with early lesions or from patients who literally "dropped dead." This permitted study of the primary process uncomplicated by secondary reactions. These studies demonstrated that the standard lesion in coronary sclerosis is atherosclerosis; that the lesions arise from the entrance of lipoids into the subendothelial layer of the intima and from their phagocytosis by cells referred to herein as lipoid cells. In the young the presence of these lipoid cells stimulates growth of fibrous tissue. As a result, the standard picture in coronary disease in the young is fibrosis with narrowing of the lumen of the artery. Death is usually due to thrombosis. In the old the presence of lipoid cells does not stimulate fibrosis. The cells accumulate in large masses, the nutrition of which becomes inadequate. Necrosis and autolysis result in liquefaction of the cell masses, and atheromatous "abscesses" result. Death is usually due to the rupture of an atheromatous "abscess" into the lumen. It is possible to reproduce the lesions of coronary sclerosis in experimental animals by feeding the lipoid cholesterol, which makes up most of the lipoid content of atherosclerotic lesions. The lesions, natural and experimental, are primarily intimal and lipoid. Lesions of the elastica and media are secondary. Inflammatory reactions are late phenomena following necrosis and are not an essential part of the picture. Atherosclerosis is a disease due to disturbances in the cholesterol metabolism and belongs with the other metabolic diseases: diabetes (carbohydrates), gout (purines) and obesity (fats).   FROM THE AUTHOR'S SUMMARY.

ACUTE EOSINOPHILIC LEUKEMIA. D. J. STEPHENS, Am. J. M. Sc. **189**:387, 1935.

A patient with acute eosinophilic leukemia is described. The clinical and pathologic features of the disease are similar to those observed in other types of

acute leukemia except for the type and maturity of the affected cell group. Evidence of extramedullary production of eosinophils is presented (a high proportion of myelocytes to adult eosinophils and large numbers of immature mononuclear cells in the spleen, lymph nodes and portal spaces of the liver). Lack of adequate neutrophilic myelopoiesis was reflected in the scarcity of neutrophilic granulocytes in the necrotizing inflammatory exudates, which were composed chiefly of adult eosinophils.

FROM THE AUTHOR'S SUMMARY.

THE HEMOPOIETIC TISSUES IN HEMOPHILIA. R. P. CUSTER and E. B. KRUMB-HAAR, Am. J. M. Sc. **189**:620, 1935.

Three fatal cases of hemophilia with necropsy are reported: in one death occurred from uncomplicated hemorrhage; in one from appendicitis and hemorrhage, and in one from fulminating pneumonia. The hematopoietic tissues all showed normal regenerative ability; in the first two cases this was predominantly erythroblastic and in the third it was leukoblastic. All three showed a marked increase of megakaryoblasts and megakaryocytes in the bone marrow, indicating a relationship of the blood platelets to the hemophilic process. J. H. Wright's observation of platelet formation in the sinusoids of the bone marrow from intruding pseudopods of megakaryocyte cytoplasm was supported by the present findings.

FROM THE AUTHORS' SUMMARY.

ETIOLOGIC AND PATHOLOGIC FACTORS IN POLYCYTHEMIA VERA. P. REZNIKOFF, N. C. FOOT and J. M. BETHEA, Am. J. M. Sc. **189**:753, 1935.

Of 134 patients suffering from polycythemia vera about 48 per cent were Jews born in eastern Europe. The records of these patients were obtained from six institutions in which the average incidence of members of this racial and national group was under 10 per cent. Seven specimens of bone marrow from patients with polycythemia vera showed distinct capillary thickening, probably fibrosis, and 6 of these showed in addition marked subintimal and adventitial fibrosis of the subarteriolar capillaries, arterioles and arteries. Of 62 control specimens only 3 of 5 specimens of agranulocytic bone marrow showed slight thickening of capillaries, probably due to edema. The rest showed no change from the normal. In the cases of general arteriosclerosis or arteriolosclerosis medial fibrosis was evident. The vascular changes, especially those of the capillaries, of the bone marrow in patients with polycythemia vera suggest the possibility that these lesions may result in anoxemia of the bone marrow with compensatory or excess compensatory polycythemia.

FROM THE AUTHORS' CONCLUSIONS.

THE MYOCARDIAL ASCHOFF BODY. L. GROSS and J. C. EHRLICH, Am. J. Path. **10**:467 and 489, 1934.

The authors have investigated the clinical histories and anatomic material in seventy cases of uncomplicated rheumatic fever in which Aschoff bodies were present in the myocardium. They suggest a classification of Aschoff bodies based on the appearance and distribution of the collagen, argentophil fibers, cell cytoplasm and cell nuclei. This classification includes seven types of Aschoff body, which apparently bear some relation to the life cycle of the lesion. Each type is described and is considered to possess sufficient characteristic features to identify it as an Aschoff body specific for rheumatic fever.

It appears that the Aschoff body passes through three stages in development. The earliest phases, represented by the small cell coronal body and the reticular body, have been found to occur up to the fourth week after the onset of the illness. The middle phases, represented by the large cell coronal, the syncytial coronal, the mosaic and the large irregular cell polarized Aschoff body, have been found to occur between the fourth and the thirteenth week after the onset of the

illness. The late phases are represented by polarized Aschoff bodies, which occur from the ninth to the sixteenth week after the onset of the illness, and subsequently by fibrillar Aschoff bodies, which occur after the thirteenth week. The earliest specific lesions are apparently influenced in their response by the reactivity of the tissue, depending on whether there has or has not been a previous attack of rheumatic fever, and also by the state of the collagen present in the interstices between the myocardial bundles. As a consequence, the evolution of these lesions may follow one of two main courses, determined by the initial lesion. The latter may occur in the form of the reticular or of the small cell coronal Aschoff body. The final phases of the life of the Aschoff body are common to both main courses. Dividing the material into four groups representing different clinical courses, there appears to be some change both in the incidence of the types of Aschoff bodies in the myocardium and in their localization. The findings reported here, however, can by no means be considered as furnishing sufficient statistical evidence on which to base final conclusions. That the tempo of the life cycle may be considerably faster or slower than has been described in this report seems probable. Some of the stages in the life cycle may be absent in some cases, abbreviated in others, or, indeed, may appear in the reverse order from that which we have suggested. These facts can be determined with greater accuracy only after a much more extensive series of cases has been examined and, in the last analysis, must await confirmation by the transmission of this disease to animals, attempts at which have thus far been unsuccessful. It is hoped that further studies will be made in order that some of these interesting relations may be placed on a firmer footing.

FROM THE AUTHORS' SUMMARIES.

THE PARATHYROID IN HYPERPARATHYROIDISM.   B. CASTLEMAN and T. B. MAL-
LORY, Am. J. Path. **11**:1, 1935.

The pathologic findings in the parathyroid glands in hyperparathyroidism may be divided sharply into two types: hyperplasia and neoplasia. The hyperplasia is characterized by diffuse uniform involvement of all the glandular tissue. It occurs, however, in two forms: a water-clear type and a much rarer chief cell type. A localized tumor of a single gland, part of a gland or rarely parts of two glands is more logically to be regarded as neoplasia. A roughly quantitative relation between the size of the enlarged glands and the degree of hyperfunction exists. The histology of parathyroid tumors provides confirmatory evidence for the monophyletic theory of the origin of the various cell types. Glycogen, albeit in minute amounts, is always present in functioning parathyroid tissue, and the concept of the oxyphilic cell as an inactive involution product receives support from a study of the adenomas.   FROM THE AUTHORS' CONCLUSIONS.

HEPATIC INFARCTION.   H. LUND, H. L. STEWART and M. M. LIEBER, Am. J. Path.
11:157, 1935.

Seven cases are described which illustrate the red, the pale and the organizing phases of the hepatic infarct. An instance of what is possibly a healed infarct is described also. Twenty cases of hepatic infarction reported in the literature are summarized.

LESIONS OF THE CORONARY ARTERIES IN RHEUMATIC FEVER.   L. GROSS, M. A.
KUGEL and E. Z. EPSTEIN, Am. J. Path. **11**:253, 1935.

A description has been presented of lesions in the main coronary arteries and their branches occuring in active and inactive rheumatic fever, together with a statistical indication of the frequency of their occurrence, the sites of predilection and a comparison of the findings with those in normal controls. The lesions in the myocardial branches have been classified under a number of different headings. Under four of these the lesions are similar to the evolutionary changes corresponding to advancing age found in the normal controls. Several of the remaining

lesions are so peculiar in their structure as to suggest that they may be specific. This observation, however, cannot be accepted as final without an extensive search for similar lesions in other diseases affecting the myocardium. A discussion is given of the mechanism concerned in the development of these lesions, of their possible relation to the development of the arteriosclerotic process and of their clinical significance.

FROM THE AUTHORS' SUMMARY.

ENDOMETRIOSIS OF THE UMBILICUS. C. V. WELLER, Am. J. Path. **11**:281, 1935.

With over seven hundred cases described in medical literature the umbilicus can no longer be considered a rare location for endometriosis. Smooth muscle is commonly not present in endometriosis of the umbilicus and is not an essential element in these formations. The term "adenomyoma" is altogether inappropriate. The sweat glands, which have been mentioned repeatedly in connection with umbilical endometriosis, have no part in the genesis of the condition. The origin of umbilical endometriosis is explainable only with the greatest difficulty under the theories that it may result from implantation or metastasis but is readily understandable under the various modifications of the serosal theory.

FROM THE AUTHOR'S CONCLUSIONS.

THE ECTOPIC DECIDUAL REACTION AND ITS SIGNIFICANCE IN ENDOMETRIOSIS. C. V. WELLER, Am. J. Path. **11**:287, 1935.

The subserous stromal cells must possess pluripotentiality in differentiation. Under the influence of suitable but dissimilar stimuli either the "cytogenic" stroma of endometriosis or the decidual reaction may result. If the former develops, it in turn may subsequently be induced to become decidual. In the decidual reaction the mesothelial cells do not appear to take any part. They lie apparently unchanged on the masses of the decidual cells. While not properly germane to the subject of this paper, it must be assumed that the surface mesothelial cells likewise possess latent potentiality in differentiation and that from them, particularly when they are entrapped in adhesions or in scar tissue, and again when they are under the stimulus of a hormone, the epithelial elements in endometriosis are derived.

CYSTIC DISEASE OF THE KIDNEYS. E. T. BELL, Am. J. Path. **11**:373, 1935.

Polycystic kidney is found once in about every five hundred necropsies, and in from 5 to 10 per cent of the cases it is unilateral. In the autopsy service of the department of pathology of the University of Minnesota about one third of the cases were observed in infants, the majority of whom were stillborn. The disease is always congenital. There are relatively few persons between infancy and the age of 25 years who show this condition clinically. One may distinguish a surgical type in which the patient presents symptoms and signs referable to one kidney, viz., pain, tumor, hematuria, infection and so on. In the medical type the symptoms are those of acute or chronic renal insufficiency, and the functional disturbances correspond to those of contracted kidneys. The attacks of hematuria are, however, distinctive. Edema is rarely prominent, and cardiac failure is unusual. The systolic blood pressure is 150 mm. of mercury or higher in over 50 per cent of the cases that have been reported, and hypertension is somewhat more frequent in advanced than in early stages of the disease. Cardiac hypertrophy often develops but is much less pronounced than in primary hypertension. Retinal changes of the hypertensive type may be found, especially in those with very high blood pressure. Some patients live many years after symptoms have developed. When the renal reserve is low, i. e., in advanced cases, pregnancy causes typical nephritic toxemia, but causes no disturbance when the renal reserve is good. There is abundant evidence that polycystic renal disease has a strong hereditary tendency. The pyelogram is of great diagnostic value when the diagnosis is otherwise difficult. In the new-born group the outstanding structural changes are the

numerous cysts, hypoplasia of the parenchyma, i. e., a great reduction in the number of nephrons, and an excessive amount of interstitial connective tissue. The numerous "glomerular" cysts are interpreted as vestigial structures derived from the first three or four generations of tubules. In the subclinical group there is abundant renal parenchyma between the cysts, while in the clinical group the parenchyma may be reduced to a few small scattered islands. The progressive atrophy of the parenchyma is brought about chiefly by continuous expansion of the cysts. Arterial disease plays a minor rôle in this process, except in the occasional case in which true primary hypertension is superimposed on the cystic disease. The arteries usually show marked intimal thickening, which is attributed chiefly to disuse atrophy but partly to hypertension. Medial fibrosis in the arteries is explainable on the basis of age. The arterioles show no marked intimal disease except when primary hypertension is a complication. However, they often show marked medial fibrosis. This process is not true arteriolosclerosis. Kampmeier's theory of the origin of the cysts is favored. One case is described in which compensatory dilatation of persistent tubules in a hypertensive contracted kidney caused it to resemble the true congenital cystic kidney.

FROM THE AUTHOR'S SUMMARY.

SIMPLE SILICOTIC PROCESS OF THE LUNG. F. W. SIMSON, J. Path. & Bact. **40**:37, 1935.

A technic for the construction of models of discrete simple silicotic nodules with their anatomic relations is described. From these and from serial sections of the nodules the following observations have been made: In the slowly developing type of simple silicosis as seen in the lungs of the Witwatersrand miner, Transvaal, South Africa, the discrete nodule is a sharply defined lesion involving a limited area of tissue in relation to the respiratory bronchioles and the proximal part of their continuation, the alveolar ducts. There is no evidence of "beading" of the larger blood vessels and bronchi from proliferative changes in the related lymphoid tissue. The silicotic nodule is composed of a mass of pigmented loose diffuse fibrous tissue and one or more whorled hyaline islets, which are usually situated in the angle formed where a terminal bronchiole divides into two respiratory bronchioles. The latter pass through the peripheral cellular zone of fibrosis surrounding the islets, and at this point usually show definite stenosis of varying degrees. The terminal air passages are accompanied by branches of the pulmonary artery but not by branches of the corresponding vein. Parts of these terminal arteries are incorporated in the fibrosis of the silicotic nodules but show, as a rule, no evidence of stenosis. The alveolar tissue in the immediate neighborhood of the discrete silicotic nodule almost invariably shows well marked emphysema.

FROM THE AUTHOR'S SUMMARY.

THE INCIDENCE OF THROMBOSIS AT NECROPSY. H. BELA, Virchows Arch. f. path. Anat. **292**:629, 1934.

The necropsies in the city hospital at Kiel, Germany, for the years 1913 to 1933 were subjected to statistical analysis to determine the correctness of the assertion that the incidence of venous thrombosis has increased since the World War. In 6,581 necropsies thrombosis was encountered 926 times (14.07 per cent). Pulmonary embolism was observed in 9.72 per cent of the necropsies. Beginning in 1919 there was a steady increase in the number of cases of thrombosis, the maximum being reached in 1928 with a figure 8½ times the average for the years preceding 1919. Since 1928 there has been only a very slight decrease. Thrombosis occurred about equally in the two sexes and was more frequent after 45 years of age. Thrombosis occurred with very great preponderance in the veins of the lower extremity and of the pelvis. Infection was the most constant single factor, with disease of the circulatory system and neoplasia ranking next. In the pulmonary embolism resulting from thrombosis the right lung was involved more often than the left and the lower lobes more often than other parts of the lungs.

O. T. SCHULTZ.

Arteriosclerosis in Sweden. Helge Sjövall and Gunnar Wihman, Acta path. et microbiol. Scandinav., supp. 20, 1934, p. 1.

This study is the Swedish contribution to the conference on arteriosclerosis under the auspices of the International Society for Geographic Pathology. The material comprises 1,075 cases from Stockholm and 305 from Lund. Macroscopic and microscopic studies were carried out on the most important arteries. No marked differences were noted between the two groups as concerns severity and frequency of arteriosclerotic changes. In Lund the women showed a somewhat greater incidence of arteriosclerosis than the men, owing perhaps to more strenuous work. The most severe arteriosclerotic changes were found in the abdominal aorta. The arch of the aorta was second in order of frequency and extent of involvement. In the coronary arteries the descending branch of the left coronary artery is most often involved, and men show earlier and more severe changes in this vessel than women. The abdominal arteries showed a comparatively low incidence and slight change, the splenic artery being most severely involved.

An increase in the average weight of the heart was noted in association with the arteriosclerotic process. Fat persons had a more severe type of arteriosclerosis than thin persons. Tuberculous patients of middle and advanced ages showed slight arteriosclerotic changes. Gallstones and arcus senilis were associated with arteriosclerosis of more than average severity. Only mild arterial changes were found with cancer.

Microscopically, the arterioles of the spleen, liver, pancreas and kidneys were particularly examined. Renal arteriolosclerosis was associated with a more severe type of macroscopic arteriosclerosis.

The lipoid deposit in the intima was studied in 290 aortas. Lipoidosis appears to develop in a regular order. The intracellular deposits of lipoid occur first, next the extracellular deposit in lamellar arrangement, then a mixed form with coarse lipoid deposits in the cells, and finally a typical atheroma. The slightest macroscopic change was associated with intracellular lipoid deposits.

Jacob Klein.

Microbiology and Parasitology

Group Infection and Immunity During a Scarlet Fever Epidemic in a Boys' School. B. Zuger, Am. J. Hyg. **21**:588, 1935.

An epidemic of 12 cases of scarlet fever in a school population of 325 boys was studied. During the year of the epidemic twice as many cases of acute tonsillitis occurred as during a similar period of the year before when an epidemic of influenza was present. Also there were 6 cases of rheumatic fever as compared with 1 case the year before. Seven strains of Streptococcus haemolyticus were isolated from boys with tonsillitis and 3 from healthy carriers. The toxin produced by 1 strain from each of these groups was subjected to neutralization tests. The reactions in both instances were similar to those of Streptococcus scarlatinae. The carrier rate in the healthy population during the epidemic varied from 6 to 20 per cent and two months after the epidemic was still 19 per cent. However, the actual numbers of hemolytic streptococci found in the individual cultures two months after the epidemic were much smaller than during the epidemic. The titer of antistreptolysin was more often elevated in the cases of tonsillitis and sore throat without a rash than in the cases of clinical scarlet fever. It was not elevated to any extent in healthy carriers or in the general population exposed to the epidemic. During the two month period of the epidemic half of the boys who had positive reactions to the Dick test at the beginning gave negative reactions toward the end without having contracted clinical scarlet fever.

From the Author's Summary.

Softening of the Caseous Tubercle and Its Results. E. R. Long, J. A. M. A. **104**:1883, 1935.

Tuberculosis in its epidemiological aspects has its source in a specific pathologic phenomenon: softening of the caseous tubercle.

This phenomenon has long been recognized but not commonly identified as a specific pathologic process, distinct from caseation.

The significance of softening of the caseous tubercle for epidemiology lies in the fact that associated with it is an enormous multiplication of tubercle bacilli. The latter are commonly hundreds or thousands of times as numerous in the semiliquid contents of softening caseous nodules as in the necrotic walls of old cavities.

Three types of case (illustrated by reports in this article) may be distinguished in a general way on the basis of the number of bacilli in the softening regions: a chronic type with a moderate concentration of bacilli in the softening lesions, an intermediate type and an acute type with vast numbers of bacilli.

The fundamental nature of the process of softening is still unknown. It is not equivalent to suppuration. Attempts to put it on an allergic basis have not been entirely successful.

The softening tubercle should receive more clinical consideration. Successful treatment of tuberculosis by collapse of the lung owes its favorable outcome as much to the prevention of drainage of liquefying tubercles as to the obliteration of large cavities. Lung collapse improperly applied, particularly with excessive pressure, even when obvious cavities are obliterated, may result unfavorably through expulsion of highly infective liquefying matter into tributary bronchioles. The most appropriate collapse, as far as the softening tubercle is concerned, is that which stops motion of the lung and partially or completely obliterates the small bronchiolar outlets from the liquefying masses.

From the Author's Summary.

Toxic Serum Extracts of Hemolytic Streptococci. J. T. Weld, J. Exper. Med. **61**:473, 1935.

The same growth of the hemolytic streptococcus may be subjected to extraction six times in two days with untreated inactivated serum with no loss in potency of the later extracts if it is kept frozen solid during the night between the extractions. The serum-extracted toxins of the hemolytic streptococcus can be preserved without deterioration for at least six months if kept frozen solid. No toxins stronger than those containing 10 units per cubic centimeter for mice have been prepared. Reasons for thinking that this is due to saturation of the serum with the toxin at this point are given. Half saturation with ammonium sulphate precipitates practically all of the hemotoxin in a preparation. Serum extracts were made from strains of the hemolytic streptococcus other than the Gay strain, and attempts were made to correlate the virulence and the production of toxin from each strain. No such correlation could be established. The principal pathologic observation in mice inoculated with the serum-extracted toxin of the streptococcus is a marked degeneration of the tubular epithelium of the kidney.

From the Author's Summary.

Survival of Encephalitis Virus (St. Louis Type) in Anopheles Quadrimaculatus. L. T. Webster, A. D. Clow and J. H. Bauer, J. Exper. Med. **61**:479, 1935.

Mosquitoes of this species, fed on mice in the blood stream of which encephalitis virus (St. Louis type) is present, take up and retain the virus for the duration of their lives. The titer of the virus in the mosquitoes four hours after they have become engorged from feeding on mice with a maximum infection of the blood stream represents about 10,000 lethal mouse intracerebral doses per mosquito.

This titer drops during the following two weeks to about 100 lethal doses per mosquito, but from the third week to the death of the mosquito it usually increases to approximately the original level and remains there. The titer of the virus in mosquitoes which have become engorged on mouse blood containing smaller quantities of virus exhibits the same drop and subsequent rise to the original level. The virus-containing mosquitoes did not infect mice or monkeys by biting.

FROM THE AUTHORS' CONCLUSIONS.

HEMOLYTIC STREPTOCOCCUS OF HUMAN ORIGIN. H. K. WARD and C. LYONS, J. Exper. Med. **61**:515 and 531, 1935.

Four common variants of the hemolytic streptococcus of human origin have been described. These have been designated the F, M, attenuated M, and C variants. Only the F and M variants have been isolated from the blood stream in streptococcic infections. Only the M has any primary virulence for the mouse. Both these variants resist phagocytosis in human blood under suitable conditions, and this appears to be a reliable test for human virulence. The attenuated M variant, found only in laboratory cultures, has a capsule as well developed as that of the virulent variant, and yet does not resist phagocytosis. The C variant has no capsule and is readily phagocytosed. It appears to correspond to the avirulent variant in other species. An attempt has been made to correlate these four variants with those already described in the literature. The application of the findings to the problem of virulence has been discussed.

An antiserum which specifically protects mice against a virulent culture (M variant) of the hemolytic streptococcus contains specific opsonin. Phagocytosis of the organisms can be observed in the peritoneum of the protected mouse. An antiserum prepared by injecting the living M variant into an animal specifically opsonizes both the F and M variant of the strain. Evidence is presented which indicates the probable identity of the specific opsonin and the anti-M precipitin of Lancefield. Agglutination appears to be dependent on a different antibody. It is possible to type the hemolytic streptococci by means of specific opsonins, and the opsonic method has certain advantages over agglutination, precipitation and mouse protection tests. It is evident from what little has been done that there are many types. The serum of infants contains no opsonin for the virulent hemolytic streptococcus, but the serum of adults may contain specific opsonins for certain strains. Inasmuch as no opsonins were demonstrable in two polyvalent antibacterial serums the possibilities of therapeutic transfusion are discussed.

FROM AUTHORS' CONCLUSIONS.

ISOLATED OUTBREAK OF EPIDEMIC MENINGITIS. G. RAKE, J. Exper. Med. **61**:545, 1935.

The investigation of this isolated epidemic of meningococcic meningitis at a camp of the Civilian Conservation Corps gave an opportunity to examine the state of carrier in contacts carrying what were presumably virulent epidemic strains of the organism. With the aid of Miller's technic for enhancement of the demonstrable virulence of meningococci for mice it proved possible to test the virulence of the strains isolated from carriers at Camp Rusk. These results were consistent despite the interval of from three to four weeks between the isolation of the strains and the titrations of their virulence. Strains of type I were found to have high virulence, while the virulence of strains of type II was moderately high but definitely less than that of strains of type I, and atypical strains and strains of Neisseria catarrhalis isolated from carriers showed very low virulence. The question of the precise nature of the state of carrier was investigated. No evidence has been obtained yet as to the existence of a relationship between pharyngitis, coryza or disease of the upper respiratory tract and the presence and degree of the state of carrier. This is unlike the situation with regard to carriers of pneumococcus. On the other hand, it has proved possible to demonstrate reactions

within the body to the meningococci in the nasopharynx, consisting of the formation of agglutinins and protective antibodies in the blood serum; 32.3 per cent of the serums of carriers of type I and 60 per cent of those of carriers of type II showed moderate or good agglutinins for the homologous organisms and 80 per cent of the serums of carriers of type I and 40 per cent of those of carriers of type II showed moderate or good protective antibodies against virulent homologous strains. No idea could be obtained as to the relationship of the presence or absence or of the degree of the serologic reaction to the duration of the carrier state.

FROM THE AUTHOR'S SUMMARY.

ENCEPHALOMYELITIS WITH MYELIN DESTRUCTION EXPERIMENTALLY PRODUCED IN MONKEYS. T. M. RIVERS and F. F. SCHWENTKER, J. Exper. Med. **61**:689, 1935.

Repeated intramuscular injections of aqueous emulsions and alcohol ether extracts of sterile normal rabbit brains in some manner produced pathologic changes accompanied by destruction of myelin in the brains of seven of eight monkeys (Macacus rhesus). Eight control monkeys remained well. Cultures from the involved brains remained sterile, and no transmissible agent was demonstrated by means of intracerebral inoculations of emulsions of bits of the brains into monkeys, rabbits, guinea-pigs and white mice.   FROM THE AUTHORS' SUMMARY.

RABBIT POX WITH ESPECIAL REFERENCE TO EPIDEMIOLOGICAL FACTORS. H. S. N. GREENE, J. Exper. Med. **61**:807, 1935.

A devastating epidemic of rabbit pox in a breeding colony was studied with especial reference to factors of epidemiological significance. The evidence obtained indicated that the epidemic originated among animals inoculated with vaccine virus and that the infection was spread to the breeding colony by caretakers. The epidemic began insidiously with atypical cases of visceral disease followed by typical cases of pox and terminated as a mild cutaneous disease with scattering monosymptomatic disorders of various kinds, difficult to recognize as cases of pox infection. An analysis of data concerning the health and functional efficiency of the population and the immunity of exposed animals showed that the epidemic of rabbit pox was the terminal event in a series of progressive disorders which began fully a month before the first case of pox occurred. In like manner, the terminal decrease in the severity of the disease and the eventual termination of the epidemic appeared to be referable to an improvement in the condition of the population rather than to a specific immunity acquired by exposure to the infection.

FROM THE AUTHOR'S SUMMARY.

MULTIPLICATION OF PSEUDORABIES VIRUS IN THE TESTICLE TISSUE OF IMMUNIZED GUINEA-PIGS. E. TRAUB, J. Exper. Med. **61**:833, 1935.

Pseudorabies virus was cultivated in vitro in washed testicle tissue from immune guinea-pigs, and evidence was thus procured that the testicle cells themselves had not become immune to pseudorabies. The rate of multiplication of the virus was considerably greater in cultures made with normal guinea-pig testis than in cultures made with immune testis. The reason for this may be that even by repeated washing the immune tissue could not be completely freed from fluid antibodies, and that the remaining antibodies somewhat inhibited the multiplication of the virus.

FROM THE AUTHOR'S SUMMARY.

"ROUGHNESS" IN STREPTOCOCCUS CULTURES FROM ENDOCARDITIS. R. TUNNICLIFF and C. WOOLSEY, J. Infect. Dis. **56**:116, 1935.

Eighty-one per cent of the streptococcus cultures from the blood of patients with subacute bacterial endocarditis belonged to the Streptococcus viridans group.

No one form of colony was demonstrated in these strains. Eighty-four per cent of the cultures showed signs of "roughness" either morphologically or colonially or both. The observations suggest that the rough element of streptococcus cultures may be an essential factor in the production of endocarditis.

FROM THE AUTHORS' SUMMARY.

BACILLARY DYSENTERY IN INFANTS AND CHILDREN. G. A. DENISON and G. DE HOLL, J. Infect. Dis. **56**:124, 1935.

The acute diarrheas of infancy should primarily be divided into (1) gastro-intestinal infections almost invariably due to dysentery bacilli and (2) gastro-intestinal disturbances of function from numerous causes. Before present classifications can be improved, bacillary dysentery must be completely removed from the other types. Infectious diarrheas are bacillary dysentery and should be so designated. Loose clinical terms are not used with uniformity and are confusing. Diagnosis should be supported by examination of the stools microscopically for pus and chemically for blood. The finding of these, with a few very obvious exceptions, always means bacillary dysentery. More reliable data are needed, however, as to the frequency with which blood and pus may be absent in the course of mild dysenteric infections. In investigative work equal consideration must be given clinical, physiologic and bacteriologic phases. In the course of a study of 35 cases of infectious diarrhea in children and infants 159 cultures showing fermentations characteristic of dysentery bacilli were isolated; 142 of these were tested with antiserums for stock strains and 116 found agglutinable. Of 72 agglutinable cultures tested by absorption of agglutinins in antiserums 53 were identified by complete and 15 by partial absorption. The isolated strains corresponded to Y (Hiss), Mt. Desert, WX, V and Sonne. An isolated strain (1-6) was identified by agglutinin absorption with 15 strains from 7 patients. Its antiserum was not appreciably affected by stock strains. It appears to be a member of the Flexner group though not identical with any of the 15 stock strains studied. Dysentery bacilli (Flexner group and Sonne) were isolated from 26 patients with infectious diarrhea (74 per cent). From each of 2 patients 2 different strains were recovered. Dysentery bacilli were recovered from 63 per cent of the stools cultured during the first five days of illness. After the fifth day the chances for recovering the organisms rapidly diminished even though the majority of the stools continued to show blood and pus for fifteen days longer. FROM THE AUTHORS' SUMMARY.

EFFECT OF SPLENECTOMY ON A LATENT INFECTION, EPERYTHROZOON COCCOIDES, IN WHITE MICE. J. MARMORSTON, J. Infect. Dis. **56**:142, 1935.

In many strains of white mice splenectomy is followed by the appearance of ringlike piroplasmic bodies (Eperythrozoon coccoides) on the red cells and in the serum of the peripheral blood. The infection is latent in these strains of mice. The bodies appear after an average incubation period of from six to seven days, and the average period during which the infection is observed is twenty-three and a half days. The height of the infection is reached about the eleventh day. In 15 per cent of the instances Bartonella muris is associated with E. coccoides, but the Bartonella infection appears toward the end of or after the disappearance of the Eperythrozoon infection. Suckling mice of noncarrier stock are not infected with E. coccoides, and splenectomy in these mice is not followed by the appearance of the infection. The injection of blood containing E. coccoides into normal suckling mice does not cause manifestations of the infection; nevertheless, subsequent splenectomy in these mice is followed by the appearance of E. coccoides in large numbers within from twenty-four to forty-eight hours, and the disease may be transmitted to splenectomized adult white mice of noncarrier stock.

FROM THE AUTHOR'S SUMMARY.

TECHNICAL ERRORS IN STUDIES OF BACTERIAL VARIATION. W. L. HOLMAN and
A. E. CARSON, J. Infect. Dis. **56**:165, 1935.

Studies in metamorphoses of bacteria should not disregard the well established
principles of pure cultures, and all the various sources of error should be adequately
eliminated before far reaching theories are elaborated in this field. Analogies are
often dangerous and are particularly so in this instance. It has been shown that
complete sterilization of mediums requires careful attention. The spores of some
aerobes may withstand 120 C. for at least an hour in the autoclave or 100 C. for
one and one-half hours in the Arnold sterilizer if protected by such a substance as
petrolatum when the same spores in broth are destroyed at these temperatures.
Many bacteria show morphologic alterations of a temporary nature, and strepto-
cocci, spore-bearing aerobic bacilli and various anaerobes are particularly prone to
do so. Bacterial association affects the growth of mixed cultures. Streptococci
were found capable of inhibiting the growth of spore-bearing aerobic bacilli. These
bacilli, moreover, could retain in their colonies streptococci or other bacteria which
may readily be overlooked. Inoculation of animals in such studies brings with it
a number of chances for contaminating the cultures used. Extraneous bacteria
may enter the inoculated area from the site on the skin or from other sources such
as the respiratory and intestinal tracts in natural infections or agonal or post-
mortem invasions. An analysis of Evans' reports on the metamorphoses of strepto-
cocci into spore-bearing rods indicates that these chances of error have not been
controlled. We have given more probable and simpler explanations for her findings
on the bases of mixed cultures, contaminations and other technical errors. There
is need today of a more conservative and critical attitude in these problems of
bacterial dissociation since there is a growing tendency to accept uncertain evidence
as if it were fundamentally established. FROM THE AUTHORS' CONCLUSIONS.

DISSOCIATION OF THE TUBERCLE BACILLUS. A. SAENZ and L. COSTIL, Presse méd.
42:1827, 1934.

This study of the dissociation of avian, human and bovine tubercle bacilli and
of paratubercle bacilli tends to throw doubt on their specificity. The authors sug-
gest that all varieties of the tubercle bacillus may have a common origin and
have become specialized after adaptation to different species. FRED STERN.

INFECTION WITH SPIROCHAETA PALLIDA THROUGH COHABITATION AND THROUGH
THE PLACENTA. W. SEIFFERT, Ztschr. f. Immunitätsforsch. u. exper. Therap.
83:386, 1934.

Mice were infected with spirochetes. After varying intervals they were exposed
to mice of the opposite sex. The syphilitic infection was not transferred, and
spirochetes were not found in the offspring or in the placentas. The infection
had a distinct inhibitory effect on the fertility of the mice. In female rabbits
similarly infected, the placentas contained spirochetes but the offspring was free.

I. DAVIDSOHN.

MUCOID VARIANTS OF BACTERIUM PARATYPHOSUM B. T. M. VOGELSANG, Acta
path. et microbiol. Scandinav. **12**:181, 1935.

Among the many hundreds of paratyphoid B strains isolated by us, we have
found only two examples of the mucoid variant. Though the mucoid form was
demonstrated in the bile in both cases, a gradual change to the S form occurred
in vitro on subculturing the strains in ox bile. This transformation took place
more quickly in the ox bile than in the other fluid mediums employed, viz., peptone
water and broth. The S forms derived from the mucoid variants showed a devia-
tion from typical S forms in that they did not form slime walls at room tempera-
ture, but otherwise reacted—as also did the mucoid forms—like typical Bact.
paratyphosum B to the different biochemical tests. From the serologic aspect the

two mucoid strains were manifestly paratyphoid B strains, having an "O" antigen in common with the S form and containing the same specific and nonspecific "H" receptors as the latter but in smaller amount.

FROM THE AUTHOR'S SUMMARY.

BRUCELLA INFECTION OF SWINE. A. THOMSEN, Acta path. et microbiol. Scandinav., supp. 21, 1934, p. 1.

The material on which the studies recorded in this monograph are based was derived from swine involved in a severe but localized outbreak of brucelliasis in Denmark in 1929-1932. In the infected herds agglutination and complement fixation with Brucella suis were positive in 39 per cent of the boars that were tested. In such boars purulent and necrotic inflammatory processes were often found in the epididymis, testis and seminal vesicles, and as Brucella suis was demonstrated in diseased as well as in normal genital organs the boar was an important spreader of the infection. The spread by boars was a conspicuous feature of the epizootic. The primary lesion in the sow consisted of whitish-yellow nodules in the deeper layer of the inner surface of the uterus (miliary brucelliasis). Brucella suis was isolated from abscesses in various places in the infected swine. The isolation was not difficult. Thomsen concludes that there is no direct relation between swine and bovine brucelliasis, and that there are essential differences between the strains of Danish porcine and bovine Brucella. Apparently no definite cases of human infection (undulant fever) developed from the swine brucelliasis.

Tumors

PRIMARY CARCINOMA OF THE LUNG. C. F. GESCHICKTER and R. DENISON, Am. J. Cancer **22**:854, 1934.

An analysis of sixty cases of primary cancer of the lung indicates that they may be classified into two groups. The more common are the cases of hilar or epidermoid carcinoma, occurring at the center of the lung and arising from the basal cell layer beneath the lining cells of the large bronchi. The rarer cases are those of lobular adenocarcinoma, occurring at the periphery of the lung and arising from the terminal ramifications of the bronchioles. Hilar or epidermoid carcinoma may be graded into three histologic forms. In the least malignant grade, occurring in patients over 50 years, squamous cells predominate. The middle grade shows a proliferation of basal and transitional cells and occurs in patients under 50 years. The most malignant grade shows masses of compact or spindle cells, is referred to as oat cell cancer, and is most common in young adults. Lobular adenocarcinoma is also divided histologically into three forms. The least malignant, most highly differentiated forms are the adenocolumnar and the adenomucoid. The average age of the patient with these forms is 40 years. The most malignant type shows a diffuse proliferation of cuboidal cells. The patients affected are most often under 40 and sometimes 30 years of age.

FROM THE AUTHORS' SUMMARY.

THE HISTOLOGIC CLASSIFICATION OF CANCERS OF THE UTERINE CERVIX AND THE RELATION BETWEEN THE GROWTH STRUCTURE AND THE RESULTS OF RADIUM TREATMENT. H. CHAMBERS, Am. J. Cancer **23**:1, 1935.

The biopsy material from 728 cases of cancer of the cervix has been examined and 500 cases have been histologically graded; 228 were unsuitable for classification. The method of grading has been based on the extent of cell differentiation and the degree of cell activity, but the general architecture of the growth has also been taken into account. The relationship of the age incidence, the duration of the symptoms and the clinical type of the growth with the histologic structure has been considered. The results of treatment have been recorded with special reference to local cure of the cancer at the primary site in relation to the histologic type; the survival rate has also been recorded for those cases which had been

under observation for more than three years. The highest percentage of local cures has been obtained in cases of the transitional type of squamous cancer grade 3, 73.8 per cent, and in cases of adenocarcinoma, 72.9 per cent, but none of the histologic grades showed a difference of more than 15 per cent in either local cure or in the number of those surviving three years. There is no evidence in this material that the adenocarcinomas are insensitive to radiation.

Studies on the Internal Organs of Mice Painted with Carcinogenic Agents. J. M. Twort and C. C. Twort, Am. J. Cancer **23**:52, 1935.

"Our studies have revealed to us that carcinogenic, therapeutic and other agents may so interfere with the general metabolism of mice that there is a profound influence, direct or indirect, on tumour development. Conversely, the presence of a tumour may so derange metabolism, especially of young animals, that cause and effect may be easily confused. It would appear that, in order to avoid errors in judgment, investigators should have a thorough knowledge of the changes likely to prevail among their animals. The observer should be in a position to discriminate between the direct and the indirect effect of a therapeutic or carcinogenic agent, with in consequence an added value to his experimental results. General debilitation and stimulation are, it would seem, the key processes to bear in mind. It is when these have been as far as possible accurately gauged, and only then, that we are in a position to evaluate changes in specific tissues. The results we have obtained up to the present time were in many respects to be expected. The general outcome of our observations is that 'balance' is the essential in keeping the animal free from malignant disease, while at the same time the latter supervenes preferentially on a healthy tissue. Additional observations will, we hope, further enlighten us as regards some of the factors responsible for this balance."

The Comparative Pathology of Carcinoma of the Pancreas, with Report of Two Cases in Mice. M. Slye, H. F. Holmes and H. G. Wells, Am. J. Cancer **23**:81, 1935.

A review of the literature indicates that the occurrence of tumors of the pancreas in all species of animals and birds is extremely rare as compared with their common occurrence in man. Among 125,000 mice of the Slye stock examined post mortem, but two primary tumors of the pancreas, both carcinomas and herewith described, have been observed. From the Authors' Summary.

On the Behavior of the Rous Tumor Virus to Freezing. J. K. Miller and H. E. Eggers, Am. J. Cancer **23**:94, 1935.

The filtrate of the Rous tumor was found to maintain its tumor-producing power after rapid freezing and thawing sixty times when these were so conducted as to avoid accompanying oxidation. There was no apparent change in its action, as determined by rate of tumor growth or by tumor morphology, when this procedure was repeated twenty times. With sixty freezings, there was delay in tumor development with marked change in tumor morphology. The filtrable agent of the Rous tumor displays a resistance to freezing and thawing greater than that exhibited by known living agents, such as bacteria or other cellular organisms. To the extent that this throws light on the nature of the tumor-producing agent, it would suggest an unorganized character. However, the possibility that the filtrate may contain organized bodies so minute as to escape the effect of sudden and repeated changes of volume cannot be absolutely precluded.

From the Authors' Summary.

Tar Epithelioma in the Sympathectomized Albino Rabbit. H. B. Raney, Am. J. Cancer **23**:98, 1935.

The fact that a majority of investigators have found an increased development of tumors after sympathectomy suggests that the sympathetic nervous system may

exert an inhibitory effect on tumor growth. The directness or indirectness of this influence is as yet a matter of conjecture; the familiar hypothesis that the vascular system acts as an intermediary has not as yet been susceptible of experimental proof.

TUMORS AND TUMOR-LIKE CONDITIONS OF THE LYMPHOCYTE, THE MYELOCYTE, THE ERYTHROCYTE AND THE RETICULUM CELL. G. R. CALLENDER, Am. J. Path. **10**:443, 1934.

A discussion of the tumors and tumor-like conditions arising from the stem cells of the lymphocytes, the granular leukocyte, the red blood corpuscle and the reticulum cell or monocyte is presented. Callender offers a classification of these conditions based on a study of the cases of the Lymphatic Tumor Registry of the American Association of Pathologists and Bacteriologists. Certain criteria for the differentiation of the conditions are given in explanation or elaboration of the tabular presentation of the classification. Certain evidence is presented that some conditions ordinarily classified as Hodgkin's disease belong to the reticulum cell group as reactive hyperplasias, aleukemic reticulocytomas or reticulum cell sarcomas. FROM THE AUTHOR'S SUMMARY.

EXTRAGENITAL CHORIOCARCINOMA IN A MAN. A. R. KANTROWITZ, Am. J. Path. **10**:531, 1934.

Kantrowitz reports the case of a man aged 22 years who had a primary teratoma of the anterior mediastinum containing choriocarcinomatous elements. At autopsy it was found that the tumor had invaded the superior vena cava and that both lungs were studded with nodules. Careful gross examination revealed no metastases in other organs or lymph nodes. The genital tract (testes, vas deferens, seminal vesicles and prostate) showed no nodules. The testicles were sectioned in 2 mm. blocks, and slides were made from each block. The slides revealed no nodules. Microscopic examination of the tumor disclosed teratomatous and choriocarcinomatous elements. Only choriocarcinoma was found in the pulmonary metastases. The testes showed no neoplastic elements. Marked interstitial cell hyperplasia of the testes was seen. These observations refute the contention of Prym and Oberndorfer, the latter writing *"dass beim Mann das Chorionepitheliom immer mit Keimdrüsengeschwülsten in Zusammenhang stehen muss"* (that in the male the chorioepithelioma must always be connected with tumors of the gonads). The Aschheim-Zondek test was positive in both the urine and the tumor tissue extracts.

MULTIPLE HEMANGIOBLASTOMA OF THE SPINAL CORD WITH SYRINGOMYELIA (LINDAU'S DISEASE). A. WOLF and S. L. WILENS, Am. J. Path. **10**:545, 1934.

A case of hemangioblastomas of the spinal cord forming part of Lindau's disease is presented. These intramedullary tumors were associated with syringomyelia and syringobulbia. The other lesions were a cystic cerebellar hemangioblastoma, congenital cysts of the pancreas and kidneys, a benign hypernephroma of the left kidney, an adrenal rest in a retroperitoneal lymph node and three paragangliomas of the left adrenal gland. FROM THE AUTHORS' SUMMARY.

PRIMARY INTRAMEDULLARY NEUROGENIC SARCOMA OF THE ULNA. J. H. PEERS, Am. J. Path. **10**:811, 1934.

Solitary intramedullary tumor of the ulna presenting the histologic structure of a perineurial type primarily occurring in bone must be exceedingly rare, the case reported here being apparently the first recorded. Estimate of the biologic character of the tumor is accordingly uncertain, but on histologic evidence it seems, unlike the neurogenic sarcoma of soft tissues, to be a tumor of low grade malignancy. FROM THE AUTHOR'S SUMMARY.

MALIGNANT TUMORS OF THE LARGE INTESTINE. L. M. LARSON and M. NORDLAND, Ann. Surg. **100**:328, 1934.

A series of 210 cases of malignant tumors of the large intestine is reviewed in this study. There was no preponderance of cases in one or the other sex. The highest age incidence was in the fifth, sixth and seventh years. The growths were located with the greatest frequency at the two extremities of the colon. More than half of the tumors were located in the rectum, rectosigmoid or lower sigmoid, and theoretically, at least, could be visualized through the proctoscope or sigmoidoscope. In about half of the cases coming to necropsy the malignant lesion was mechanically resectable by surgical methods, inasmuch as no extension or metastasis was found at autopsy. About a third of the patients presented metastases in the liver or regional glands. Practically every organ in the body was involved in metastases in this series of cases. No significant difference was noted in the incidence of metastases relative to the location of the lesion. Obstruction took place in 81 per cent of the cases. The immediate cause of death was most frequently peritonitis or exhaustion, but associated lesions such as cardiorenal vascular or pulmonary diseases, hypertrophy of the prostate or acute appendicitis contributed to the low resistance of these patients. In sixteen cases polyposis was present in localized or diffuse form, and in each one of these cases the evidence indicated that malignant change took place on a preexisting benign polyp.

FROM THE AUTHORS' SUMMARY.

TUMORS OF THE FROG "ROUSSE." J. M. PIRLOT and M. WELSCH, Arch. internat. de méd. expér. **9**: 341, 1934.

There are skin irregularities in the frog which are similar to cutaneous neoplasms. They occur singly and in groups. They are not transferable by grafting. Small subcutaneous cysts, found mostly in the male frog, can be transmitted by means of grafts of the skin. Two cases of adenocarcinoma and three of adenoma of the skin in man were traced to a single species of frog. A myxofibrochondroma could not be transplanted by grafting.

ELIZABETH McBROOM.

ARRHENOBLASTOMA. H. O. KLEINE, Arch. f. Gynäk. **157**:410, 1934.

Thirty-five cases of arrhenoblastoma of tubular structure have been reported thus far. Among the four cases that Kleine observed was one in which there was a dermoid cystoma in the other ovary. The growth of these tumors began at the hilus. Leydig's interstitial cells were demonstrable in all. The patients exhibited more or less characteristic signs of virilism. In one patient the rete of the other ovary was considerably enlarged but free from tumor cells. The development of a postoperative hyperthyroidism in one of the patients indicates the possibility of a pluriglandular disturbance. The author agrees with R. Meyer that the parent tissues of arrhenoblastoma are the rete ovarii, the medullary strands and the so-called extraglandular interstitial cells. These three epithelial formations represent heterosexual cell complexes of the ovary, which are present in small amounts in every ovary. The hypoplasia of the isosexual gonadal parenchyma seems to promote the development of the heterosexual tissues. The observations of other authors (R. Meyer) on younger women with arrhenoblastoma, in whom signs of virilism disappeared after extirpation and reappeared in case of relapse, prove a causal connection between these tumors and virilism. Three problems have yet to be solved: (1) the significance of the rete testis and of Leydig's interstitial cells for the development of the secondary male sex characters, (2) the problem whether arrhenoblastoma forms a testicular incretion, and (3) whether there are relations between arrhenoblastoma and the adrenal system.

INTRATRACHEAL THYMOMA. A. WALDOŃ, Centralbl. f. allg. Path. u. path. Anat. **60**:308, 1934.

In the body of a woman 58 years old, who had had respiratory difficulty for three months prior to death from bronchopneumonia, a soft tumor was found

attached by a pedicle to the front wall of the trachea, 3 cm. above the bifurcation. The tumor was 2.5 cm. long, 1 to 1.3 cm. wide and 1 cm. thick, and the pedicle was 6 mm. in diameter. In sagittal section the upper half of the growth contained a cyst 1 cm. in diameter, tense, with two outpouchings, and filled with blood. The tissue surrounding the cyst was mottled red while that in the lower half of the tumor was yellow. The mediastinal lymph glands were enlarged from simple hyperplasia, and the thymus was replaced by fat. The microscopic observations were as follows: The stalk was covered with ciliated epithelium and contained one central and two lateral connective tissue bands which delimited two tracts. These tracts consisted of thymic reticulum cells, in which were Hassall's corpuscles, and coursed from the tumor, between two cartilage rings, to the extratracheal tissue. The ventral upper part of the tumor was covered with ciliated epithelium which suddenly gave way to stratified squamous epithelium from 5 to 8 cells thick. The papillae underlying this epithelium were short and flat in the upper half of the tumor but were well marked in the lower half. The lower half of the back part of the tumor was partially coated with cylindric epithelium which, in some places, sent tubular glandlike outpouchings into the tumor. At the lower pole there was a mixture of cylindric and squamous epithelium irregularly coating the tumor. Internal to the fibroblastic connective tissue layer in the top half of the tumor were a cellular ground-work and regularly distributed Hassall's corpuscles. In the interior of the lower half collogenic fibers surrounded masses of squamous epithelium with centers consisting of cornified cells. These epithelial masses and strands were continuous with the covering of the tumor. The tumor, therefore, in addition to being a rare growth of thymic origin, was transformed into a carcinoma in its lower half. GEORGE RUKSTINAT.

TUMORS OF THE PERIPHERAL NERVOUS SYSTEM. H. J. SCHERER, Virchows Arch. f. path. Anat. **292**:479, 1934.

Scherer devotes seventy-five pages to a comparative histologic study of a variety of tumors of the peripheral nervous system, including neuroblastoma of the sympathetic nervous system, neuroganglioma, neurinoma, solitary diffuse overgrowths of peripheral nerves and the neurofibromatous tumors of Recklinghausen's disease. The purpose of the study was to determine the neuro-ectodermal or mesenchymal origin of neurinoma. In the ganglioneuromas there were found cellular areas that Scherer terms "neuro-ectodermal germinal centers." Some of these consisted of undifferentiated cells like those of neuroblastoma; in others, differentiation into ganglion cells was evident. Neurofibril formation in ganglioneuroma occurs independently of ganglion cells. Especial importance is attached to the neuro-ectodermal germinal centers of ganglioneuroma because the presence of similar areas in neurinoma, described for the first time by the author, establishes the neuro-ectodermal origin of neurinoma. This tumor is derived from the ectodermal supporting tissue of the nerves. The germinal tissue does not give rise to ganglion cells, and the neurinoma cannot be considered a peripheral glioma. Neurofibromatosis presents a number of problems that are not possible of solution in the present state of knowledge. In Recklinghausen's disease there is an overgrowth of both mesenchymal supporting tissue and neuro-ectodermal supporting tissue. Which tissue is primarily concerned in the overgrowth it is impossible to determine; both may be equally concerned as the result of not at present understood developmental mechanical factors. O. T. SCHULTZ.

DIFFERENTIAL HISTOLOGIC DIAGNOSIS OF INTRACEREBRAL NEURINOMA. H. J. SCHERER, Virchows Arch. f. path. Anat. **292**:554, 1934.

In a case of what is termed "rudimentary Recklinghausen's disease," there were a glioma of the pons and gliosis of the optic and olfactory nerves. In the cerebrum beneath the left ventricle were a number of small nodules that with the usual staining methods were held to be neurinomas. More careful examination of these revealed that the younger nodules were composed of fibril-forming

astrocytes and that the older and denser nodules were composed largely of fibrillated glia. The lesions were not neurinomas but astrocytic glioses or gliomas. His findings lead Scherer to define the histologic criteria that should make it possible to distinguish between a central neurinoma and a glioma. The fibrils of the neurinoma are more sharply contoured and have a straighter course than those of glioma. The neurinoma is more sharply demarcated at its periphery from the surrounding brain tissue. The palisade arrangement of the nuclei, which is the most striking feature of neurinoma and is evidence of the organoid character of the tumor, differs from the pseudopalisade arrangement not infrequently seen in a variety of other fibrillated tumors.   O. T. Schultz.

Differential Diagnosis of Neurogenic Tumors. H. J. Scherer, Virchows Arch. f. path. Anat. **292**:562, 1934.

In a preceding study of tumors of the peripheral nervous system Scherer reached the conclusion that the presence of perivascular areas of cellular undifferentiated tissue establishes the neurogenic origin of neurinoma. He describes two tumors of the mediastinum in the histologic diagnosis of which ganglioneuroma could be excluded but which could not be diagnosed as neurinoma by the usual criteria. One tumor was fibromatous or fibrosarcomatous; the other, gliomatous or more frankly sarcomatous. The denser tumor contained perivascular areas of partly differentiated tissue; the other neoplasm, very cellular perivascular areas of undifferentiated tissue. Scherer thinks that the perivascular tissue is neurogenous in origin and establishes a probable neurogenic origin of the tumros.

O. T. Schultz.

Tumor-Like Forms of Lymphogranulomatosis. A. Dudits, Ztschr. f. Krebsforsch. **40**:229, 1934.

The occasional evidences of polymorphism among the cells of the so-called lymphogranulomatoses may at times be significant of actual malignancy inasmuch as such cells may give rise to malignant tumors apparently of the reticulosarcomatous type. Two such cases are described in this article, the first tumor being primary in the intestinal wall, with relations to the mesenteric nodes but possibly originating in an intestinal follicle, and the second being a typical example of the transformation first discussed.   H. E. Eggers.

The Inheritance of Cancer. J. Körbler, Ztschr. f. Krebsforsch. **40**:271, 1934.

In connection with reports on several instances of the familial occurrence of cancer, Körbler points out that many cases of this sort may be equally well explained on a basis of actual transfer; among his series is one in which the theory of infection would meet the stated facts better than that of heredity. In still another described by him, the family had both tuberculous and cancerous antecedents, and here there was a curious separation of the two diseases in different branches of the family. As regards the relations of tuberculosis to cancer, he is of the opinion that as cancer is outstandingly a disease of unimpaired vitality, the previous presence of tuberculosis would react against the development of the former disease. The reverse of this relationship, he believes, is shown by the relation of cancer to longevity, in which there would occur long continuance of unimpaired vital function.   H. E. Eggers.

Technical

An Ultramicro Technic for Precipitin and Agglutinin Reactions. C. L. Hudson and S. Mudd, J. Immunol. **28**:311, 1935.

The capillary tube technic developed by Richards, Bordley and Walker (*J. Biol. Chem.* **101**:179, 1933) for the chemical ultramicro-analysis of minute amounts of

fluid has been adopted for the performance of certain immunologic reactions. Precipitation and agglutination tests have been made with as little as 0.1 cu. mm. of reacting material. In sensitivity and reliability this technic was found equal to standard macromethods. The apparatus required is neither expensive nor difficult to obtain and the technic is easily mastered. The obvious advantages of the ultra-micromethod are the minuteness of the quantity of fluid required and the rapidity with which a determination can be completed. An additional advantage is the opportunity for microscopic observation of the precipitation and agglutination during the course of reaction. The vividness with which the reactions may be seen contributes an appreciation of the character of these phenomena even to an observer experienced with macromethods.   FROM THE AUTHORS' SUMMARY.

A SIMPLE DIFFERENTIAL STAIN FOR THE HUMAN HYPOPHYSIS. C. SPARK, J. Lab. & Clin. Med. **20**:508, 1935.

Fixation.—Orth's fluid (Müller's fluid, 9 parts, dilute solution of formaldehyde, 1 part) is superior to fixatives containing mercuric bichloride such as Zenker's or Helly's fluid, which result in a marked brittleness of the tissue. The tissue is fixed for forty-eight hours and washed in running water for twenty-four hours.

Staining.—Paraffin sections from 6 to 8 microns thick are brought down to water. The sections are stained as follows:

1. Immerse in 0.25 per cent aqueous aniline blue for from sixty to ninety seconds. The basophil cells are stained deep blue, while the rest of the tissue is stained very faint blue.

2. Wash in tap water for one-half minute. Excessive washing will remove the dye from the basophils.

3. Immerse in Mayer's hematoxylin for ten minutes.

4. Wash in tap water for from two to three minutes.

5. Immerse in Van Gieson's mixture for from sixty to ninety seconds. The mixture is made up by adding 5 cc. of 2 per cent aqueous acid fuchsin to 100 cc. of saturated aqueous trinitrophenol. Excessive treatment with Van Gieson's mixture will result in a greenish blue coloration of the basophils.

6. Wash in tap water for one minute. Prolonged washing will wash out the Van Gieson stain.

7. Transfer sections to 95 per cent alcohol for one minute.

8. Dehydrate in absolute alcohol for from two to three minutes.

9. Clear in xylene.

By this method the various cellular elements of the human hypophysis are sharply delineated. Nuclear chromatin is stained purplish brown. The beta (basophilic) granules are stained dark blue and appear in sharp contrast to the alpha (acidophilic) granules, which stain olive green. The cytoplasm of the chromophobe cells are light grayish blue. Red blood cells stain bright yellow, which adds considerably to the ease of studying the sections, especially in glands that show congestion of the blood vessels. The colloid in the anterior lobe stains a variety of colors varying from yellowish to purple. The colloid in the posterior lobe stains light blue.

Dense collagenic fibers stain deeply with the acid fuchsin, while loose collagenic connective tissue stains lightly either with aniline blue or with acid fuchsin. The capillary walls stain sharp blue. The stained sections possess a good degree of optical translucency, which was not obtained with several of the published stains for the hypophysis.

Stained sections that are now two years old have not shown any significant degree of fading.

Book Reviews

A Textbook of Biochemistry. Edited by Benjamin Harrow, Ph.D., Associate
Professor of Chemistry, The City College, College of the City of New York,
and Carl P. Sherwin, M.D., Sc.D., Dr.P.H., Member of the Staff of St.
Vincent's Hospital, New York. Cloth. Price, $6. Pp. 797, with 52 illustra-
tions. Philadelphia: W. B. Saunders Company, 1935.

In the preface to this book the editors state: "Biochemistry, like several other
branches of science, has become so encyclopedic in its scope, that it seems an
impossible task for any one individual to write an adequate textbook. It is for
this reason that we have asked specialists in the various fields of biochemistry to
contribute the chapters constituting this book." This explains the general nature
of the book. It has been divided into thirty chapters, written by as many different
authors, all professors or investigators in the field of biochemistry.

The book covers a somewhat wider range than the usual textbook of bio-
chemistry. In the first chapter Chambers discusses the action of the living cell.
The next six chapters deal with the chemistry of carbohydrates, fats, sterols,
proteins, amino-acids and nucleic acids. Chapters 8, 9, 10 and 11, dealing with
nutrition, the vitamins, the enzymes and digestion, offer a general and comprehen-
sive discussion of these subjects. The chapter on the biochemistry of bacteria,
yeasts and molds by Clutterbuck and Raistrick is of particular interest because it
presents one of the new fields for investigation in biochemistry. Carl P. Sherwin
presents a detailed dissertation on detoxication in chapter 13. Chapters 15, 16, 17
and 18 are discussions of the blood and blood gases, respiration and animal pig-
ments. The oxidation-reduction reactions of cells are described in detail in
chapter 19. In this chapter, as in the chapter on immunochemistry, the material
presented is highly specialized and might prove somewhat detailed for the begin-
ning student of biochemistry and for the medical student.

The scope of Heidelberger's chapter is well illustrated in his summary, which
is also of interest because it indicates the present state of knowledge of the chem-
istry of antigen-antibody reactions. Heidelberger states: "In this chapter the
writer has tried to show how chemistry has made steps toward giving more definite
meaning to the concepts antigen and antibody and providing a better understanding
of the mechanism of the immune reactions in which they participate. The introduc-
tion of known chemical groups into the protein molecule, with its consequent
sensitive control of specificity, and the recognition of the large part played by poly-
saccharides in bacterial specificity have served to emphasize the essentially chemical
and ultimately minutely determinable basis of biological specificity, and have
simplified and clarified relationships and provided powerful aids for further
progress. With highly purified antibody close at hand, and with plausible theories
as to its formation, the many problems connected with antibodies should be well
on their way toward solution. With these newer aids it has been possible to obtain
strong evidence of the chemical union of antigen or hapten with antibody in multiple
proportions, and to express this union in terms of the laws of classical chemistry.
On this foundation there are now accessible new and absolute quantitative methods
which should be useful tools in the acquisition of a final complete understanding
of immune processes."

The succeeding chapters deal, for the most part, more generally with the metab-
olism of proteins, carbohydrates, fats and minerals. The chemistry of the skin,
muscle, bone, brain and urine are discussed in the latter part of the book, the
last chapter being a short discussion of hormones. In general, the articles are
well written and interesting. However, as a textbook, the result may be some-
what too detailed for the student of medicine and the beginner in the field of
biochemistry. The book might well be used for supplementary reading, for it
presents a comprehensive and highly detailed discussion of the various aspects of

biochemistry. This specialization necessitates the omission of many fundamental facts essential in a textbook for students. The bibliographies at the end of each chapter offer a wealth of material. The book is highly recommended for teachers, advanced students and investigators in the field of biochemistry.

The Spleen and Resistance. By David Perla, M.D., Associate Pathologist, Montefiore Hospital, and Jessie Marmorston, M.D., Associate in Pathology, Cornell University Medical College. Price, $2. Pp. 170. Baltimore: Williams & Wilkins Company, 1935.

The book begins with a review of the anatomy of the spleen. The major elements in its structure are derived from reticular cells or mesenchymal reticulum. In infections the structural changes, and presumably also the functions, vary according to the species and the type of infection. The large number of macrophages in the spleen indicates its capacity for phagocytosis, but it is not assumed that phagocytosis is the only or the most important factor in resistance. From an analysis of the experiments on the formation of antibodies by the spleen it is concluded that splenectomy lowers the power of the body to form antibodies. Some of these experiments failed to take into consideration the circumstance that in dogs, mice and rats splenectomy may change certain existing latent infections (those due to Bartonella, Klossiella, eperythrozoon coccoides) into active diseases. In these and other types of infection splenectomy depresses natural as well as acquired resistance in many species of animals. In many of these diseases the macrophagic tissues of the spleen are involved. Splenectomy is followed also by proliferation of macrophages in the lymph nodes, liver, marrow and lungs. This proliferation is interpreted as compensatory in nature and due to an activation from splenectomy of mesenchymal reticular cells and their derivatives everywhere in the body. The rôle of the spleen in resistance consequently is not to be ascribed wholly to phagocytosis, because it may be involved in "subtle chemical interrelationships, at present little understood." However, the importance of the spleen in natural and acquired resistance to certain bacterial and protozoan infections is now well established. The study of the spleen in latent infections by the authors and others throws a new and helpful light on experiments in splenic physiology. These experiments have resulted in contradictions and controversies because they dealt with different species and with carriers of latent infections that induce anemia when the spleen is removed. "It becomes extremely questionable, in view of these facts, that the spleen plays the rôle in hemoglobin formation previously attributed to it on the basis of studies of iron metabolism in splenectomized animals in which the presence of latent infection has not been excluded."

There is a good bibliography, also complete subject and author indexes. While this is of minor importance, perhaps, note must be made of the fact that the proper names of micro-organisms are printed sometimes in italics and sometimes in ordinary type and in either case sometimes with and sometimes without the usual initial capitalization. The monograph presents well the present understanding of the relation of the spleen to resistance to infection. It will be of value in the further study of the spleen in health and disease.

Books Received

PRÉCIS DE THÉRAPEUTIQUE ET DE PHARMACOLOGIE. R. Hazard, Professeur agrégé à la Faculté de Médecine de Paris, Pharmacien des Hôpitaux. With a Preface by A. Desgrez, Membre de l'Institut et de l'Académie de Médecine. Third edition, completely revised. Price, stitched, 85 francs; bound, 100 francs. Pp. 1257, with 34 illustrations. Paris: Masson & Cie, 1935.

ANGINES LYMPHO-MONOCYTAIRES AGRANULOCYTOSE; LEUCÉMIES LEUCOPÉNIQUES. J. Cabrazès, Professeur à la Faculté de Médecine de Bordeaux, and René Saric, Interne des Hôpitaux de Bordeaux. Price, 40 francs. Pp. 364, with 18 figures. Paris: Masson & Cie, 1935.

This book contains three chapters. The first deals with the disease or group of diseases which in the English literature are now perhaps most frequently called infectious mononucleosis. The second chapter is devoted to the conditions included under the term agranulocytosis, and the third chapter to aleukemic leukemia and allied diseases. Each chapter is a complete monograph by itself, with a select bibliography of original articles and reviews on the subject with which it deals. At the end of the book is a general subject index, which is a rather unusual feature in a French publication of this kind. The more important literature concerning the diseases under consideration is reviewed carefully, and a number of illustrative cases observed by the authors are reported in detail. The book will give the reader a clear and comprehensive summary of the present knowledge and views of the different phases and relations of infectious mononucleosis, agranulocytosis and aleukemic leukemia—all topics of actively growing interest and study at this time.

THE SPLEEN AND RESISTANCE. David Perla, M.D., Associate Pathologist, Montefiore Hospital, and Jessie Marmorston, M.D., Associate in Pathology, Cornell University Medical College. Price, $2. Pp. 170. Baltimore: Williams & Wilkins Company, 1935.